Genetic and dietary interactions in the regulation of HMG-CoA reductase gene expression

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Abstract Inbred strains of mice exhibit large genetic variations in hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. A tissue-specific genetic variation between the strains BALB/c and C57BL/6, resulting in about 5-fold higher levels in hepatic reductase activity in strain C57BL/6, was examined in detail. The activity difference between these two strains could be explained entirely by differences in hepatic reductase mRNA levels. In genetic crosses, the variation segregated as a single major Mendelian element. Surprisingly, the mode of inheritance was recessive since F_1 mice exhibited the BALB/c levels of enzyme activity. Despite the fact that the rates of hepatic sterol synthesis also differed between the strains by a factor of about five, the altered hepatic reductase expression did not significantly influence plasma lipoprotein levels. The response to a high cholesterol, high fat diet between the strains was remarkably different. Thus, in BALB/c mice, both hepatic reductase activity and mRNA levels were affected only slightly, if at all, by cholesterol feeding, while in strain C57BL/6 mice both were reduced more than 10-fold by cholesterol feeding. Several lines of evidence, including analysis of *cis*-acting regulatory elements, the nonadditive mode of inheritance, and genetic studies of the HMG-CoA reductase gene locus on mouse chromosome 13, support the possibility that the variation in reductase expression is not due to a mutation of the structural gene but, rather, is determined by a *trans*-acting factor controlling reductase mRNA levels. The variation provides a striking example, at the molecular level, of the importance of dietary-genetic interactions in the control of cholesterol metabolism.—**Hwa, J. J., S. Zollman, C. H. Warden, B. A. Taylor, P. A. Edwards, A. M. Fogelman, and A. J. Lusis.** Genetic and dietary interactions in the regulation of HMG-CoA reductase gene expression. *J. Lipid Res.* 1992. **33:** 711–725.

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3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (E.C.1.1.1.3.4) catalyzes the formation of mevalonate from HMG-CoA and is thought to be the rate-limiting enzyme for cholesterol synthesis in liver. The mevalonate is in turn incorporated into cholesterol and a variety of other isoprenoids, including the isopentenyl group of transfer RNAs, ubiquinone, dolichol, and the donor for prenylated proteins. Structurally, HMG-CoA reductase is a trans-membrane glycoprotein of the endoplasmic reticulum, composed of two identical 97-kDa subunits (1–3). The activity of HMG-CoA reductase is regulated by negative feedback mechanisms mediated by sterol and nonsterol metabolites derived from mevalonate. Thus, cholesterol feeding suppresses hepatic HMG-CoA reductase activity, whereas bile acid sequestering drugs (such as cholestyramine) and competitive inhibitors of HMG-CoA reductase (such as mevinolin) induce the enzyme (4). The feedback regulation of HMG-CoA reductase is unusually complex, involving transcriptional repression (4–6), translational regulation (7, 8), mRNA turnover (9), enzyme turnover (10–12), and enzyme activation (13). Studies with tissue culture cells have also shown the importance of a nonsterol component in the control of enzyme expression (14). In addition to the regulation exerted by diet and drugs, rat hepatic reductase exhibits a diurnal rhythm of activity that is regulated primarily at the transcriptional level (15).

As part of an effort to develop the mouse as a model for the genetic control of cholesterol metabolism and the early stages of atherosclerosis, we have studied the expression of HMG-CoA reductase among inbred strains of mice. As originally reported by Packie and Kandutsch in 1970 (16), inbred strains vary widely in the level of hepatic reductase activity and the rates of sterol synthesis. In fact, our extended survey reveals about a 25-fold range of

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PCR, polymerase chain reaction.

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hepatic reductase specific activities among inbred strains. In this report, we present data pertaining to the genetic and molecular factors determining these dramatic genetic variations, as well as their physiological consequences. The results provide a striking example of the importance of genetic-dietary interactions in cholesterol metabolism.

METHODS

Animals and diets

Female mice 2–4 months of age were obtained from The Jackson Laboratory, Bar Harbor, ME. Normal chow was Purina chow containing 4% fat. Mice were housed individually for 1–2 weeks prior to the experiment to reduce nongenetic variability resulting from any interactions between multiple mice in a single cage. To avoid differences resulting from diurnal changes in reductase activity, animals were maintained on a strict 12 h light–dark cycle and, unless otherwise noted, examined at the nadir (mid-light phase) of the HMG-CoA reductase cycle. Also, mice of BALB/cJ strain were included in all experiments to allow normalization for variability in the HMG-CoA reductase assays between separate experiments. The high fat diet was prepared by mixing the Thomas-Hartcroft diet purchased from Teklad Test Diets, Madison, WI, with Purina breeder chow (diet 5015) in a ratio of 1 part to 3 parts, respectively. The resulting diet contains, by weight, 7.5% cocoa butter, 1.25% cholesterol, and 0.5% cholic acid and has previously been characterized for its effects on lipoprotein metabolism and atherosclerosis (17–19). Mice were fed the high fat diet for 3 weeks prior to examination of HMG-CoA reductase activity and mRNA levels. For the cholestyramine-mevinolin studies, the chow diet was supplemented with 5% cholestyramine for 2 days and then 5% cholestyramine plus 0.1% mevinolin for 2 days prior to killing the animals.

Lipid and protein determinations

The hepatic and plasma total cholesterol concentrations were determined using a hexane extraction procedure (20), and triglycerides were determined by a colorimetric micromethod (21). Plasma high density lipoprotein (HDL) cholesterol levels were determined after selective precipitation of low density and very low density lipoproteins (LDL and VLDL) using heparin/manganese treatment (22). Proteins were measured by the method of Bradford (23).

Assay of HMG-CoA reductase activity

Mouse livers were homogenized at 4°C in 10 ml of buffer A (250 mM sucrose, 15 mM EGTA, 10 μM leupeptin, 5 μg/ml aprotinin, and 0.5 mM phenylmethylsulfonylfluoride, pH 7.4) with a motor-driven loose-fitting, glass-Teflon Potter-Elvejhem homogenizer, and the microsomes were prepared as previously described and stored frozen (24). The microsomes were sonicated in buffer B (0.2 M KCl, 0.16 M KH_2PO_4, 4 mM EDTA, and 10 mM dithiothreitol, pH 6.8) and the enzyme was then assayed in 250 μl of buffer B containing 2.8 μmol D-glucose-6-phosphate, 0.56 μmol NADP, 1.65 units glucose-6-phosphate dehydrogenase, 18 μmol NaCl, 75 nmol *RS*-[3-^{14}C]HMG-CoA (0.45 Ci/mol), and 0.2 mg microsomal protein. When assaying the enzyme in animals treated with the cholestyramine–mevinolin diet, microsomes were diluted appropriately to reflect the induction of enzyme activity. The [3-^{14}C]mevalonone synthesized during the assay was isolated by anion-exchange column chromatography as previously described (25).

Quantitation of mRNA

Total RNA was isolated from tissues by homogenization in guanidine thiocyanate followed by centrifugation through a cesium chloride cushion (26). Northern blots of total RNA were prepared using nylon filters and probed with hamster HMG-CoA reductase cDNA (27) generously provided by Drs. J. L. Goldstein, M. S. Brown, D. Chin, and K. L. Luskey, Department of Molecular Genetics, University of Texas Health Science Center, Dallas, TX. For hybridization, a 3.5 kb EcoRI cDNA fragment was isolated and radiolabeled with [^{32}P]dCTP to a specific activity of 1–5 × 10^9 cpm/μg by random priming. Hybridizations were performed as previously described (28). The filters were then dried and exposed to Kodak XAR-5 X-ray film. Levels of RNA were normalized by probing the same blots for constitutively expressed α-tubulin mRNA (28) and filters were washed at 65°C in 15 mM NaCl, 1.4 mM trisodium citrate, pH 8.0, 0.1% SDS.

Southern blotting and linkage analysis

Mouse genomic DNA was isolated from spleen cell nuclei as described previously (18). The strains used were AKR/J (AKR), BALB/cJ (BALB/c), C3H/HeJ (C3H), C57BL/6J (C57BL/6), NZB/BINJ (NZB), SWR/J (SWR), C57L/J (C57L), C58/J (C58), DBA/2J (DBA) and 129/J (129), and various recombinant inbred (RI) strains. Southern blots of digested DNA were prepared using nylon filters and hybridized to a 3.5 kb hamster cDNA insert described above essentially as previously described (18). Restriction fragment length variations of the HMG-CoA reductase gene were identified among inbred mouse strains and linkage analysis was carried out using recombinant inbred (RI) strains of mice and backcrosses (29).

Analysis of mouse HMG-CoA reductase promoter

A sequence for the 5' flanking region of the mouse HMG-CoA reductase gene was generously provided by Dr. Richard Lathe, INSERM, Strasbourg, France. Two primers were prepared for polymerase chain reaction (PCR) amplification and sequencing of the promoters from various strains

of mice. The 5' primer, which hybridizes about 440 bases 5' of the mouse transcription start site, has the sequence: 5' TACTTCCCCACAGATCTCTCTACTA 3'. The 3' primer, which contains four random bases at the 5' end followed by a HindIII site and 20 bases corresponding to the first intron of the mouse HMG-CoA reductase gene, has the sequence: 5' TACGAAGCTTTCCCGCCGGTCCCCAGCGTC 3'. Conditions for PCR and sequence analysis were essentially as previously described (30). The PCR products were digested with HindIII and PstI (which cuts the product about 100 bp 3' of the 5' primer) and subcloned into plasmids. The PCR products were sequenced in both orientations and a number of independent subcloned fragments were sequenced from each strain.

RESULTS

Variation in hepatic HMG-CoA reductase expression

Large genetic differences in hepatic HMG-CoA reductase specific activity were observed among inbred strains of mice maintained on a chow diet (**Table 1**). The hepatic HMG-CoA reductase activity difference between the highest and the lowest strains was about 24-fold. The results are entirely consistent with the smaller survey of Packie and Kandutsch (16). These authors also demonstrated that the differences in hepatic reductase activity were associated with corresponding differences in the rate of sterol synthesis as judged by acetate incorporation, consistent with the concept that hepatic reductase activity is rate-limiting for sterol synthesis. As pointed out in this previous study (16), nongenetic variation was reduced by killing animals at the same point in the diurnal cycle (see below). During preliminary work we observed a marked reduction in nongenetic (intrastrain) activity variability when animals were housed in separate cages for several days prior to examining activity. Thus, the coefficient of variation for hepatic reductase activity of strain C57BL/6 was about 50% with multiple (six) mice per cage but was less than 30% with a single mouse per cage (data not shown). Interestingly, the variation for reductase activity of BALB/c mice was not significantly altered whether the mice were housed six per cage or individually. A possible explanation for this genetic difference in environmental responsiveness is discussed below. The values reported in Table 1 are for mice housed individually.

TABLE 1. Levels of hepatic HMG-CoA reductase activity among inbred strains of mice

Strains	Hepatic HMG-CoA Reductase Specific Activity
C58/J	0.024 ± 0.002 (9)
BALB/cJ	0.048 ± 0.004 (20)
SWR/J	0.064 ± 0.012 (5)
A/J	0.110 ± 0.026 (5)
C57L/J	0.116 ± 0.016 (5)
129/J	0.126 ± 0.036 (5)
AKR/J	0.208 ± 0.028 (5)
C57BL/6J	0.222 ± 0.024 (10)
NZB/BINJ	0.280 ± 0.118 (4)
SJL/J	0.330 ± 0.048 (4)
C3H/HeJ	0.444 ± 0.024 (5)
DBA/2J	0.568 ± 0.162 (5)

Values are expressed as nmol mevalonic acid formed/min per mg protein ± standard error; the number of animals tested is given in parentheses.

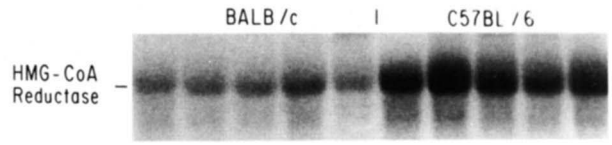

Fig. 1. Hepatic HMG-CoA reductase mRNA levels in strains C57BL/6 and BALB/c. Fifteen μg of total liver RNA was applied to each lane. RNA was denatured, electrophoresed on 1% agarose gels, transferred to nylon, and probed with ^{32}P-labeled hamster reductase cDNA, as described under Experimental Procedures. Lanes 1–5 contained RNA isolated from individual BALB/c mice and lanes 6–10 contained RNA from individual C57BL/6 mice.

This survey revealed a continuum in reductase activity among mouse strains surveyed (Table 1), suggesting that the variation results from a combination of polymorphisms affecting enzyme expression. This conclusion is supported by the finding that some but clearly not all of the variation is explained by differences in reductase mRNA (**Fig. 1** and **Fig. 2**). Thus, the strains C57BL/6 and BALB/c differ about 5-fold in hepatic reductase activity, with a corresponding difference in reductase mRNA levels. On the other hand, strain C3H has higher levels of reductase activity, but lower levels of reductase mRNA, than strain C57BL/6, and strain A has lower levels of reductase activity but higher levels of reductase mRNA than strain C57BL/6. The unusually large variation in reductase expression is somewhat surprising, particularly since the enzyme is thought to play a crucial role in the regulation of sterol synthesis. On the other hand, it is clear from biochemical studies that the enzyme is regulated at multiple levels, including transcription, translation, and turnover; thus, a variety of different types of variation could affect reductase expression. The availability of variations affecting various aspects of reductase expression provides an opportunity to examine the molecular and genetic mechanisms controlling the enzyme in vivo. We selected two inbred strains of mice, C57BL/6 and BALB/c, for detailed biochemical and genetic analyses. As shown above, the difference in reductase activity in these strains can be explained entirely by differences in reductase mRNA expression, suggesting that the variation may result from mutation of a single gene. Moreover, the strains are parents of a set of recombinant inbred strains (CXB), facilitating genetic studies. Finally, both strains have been relatively well characterized with respect to lipoprotein

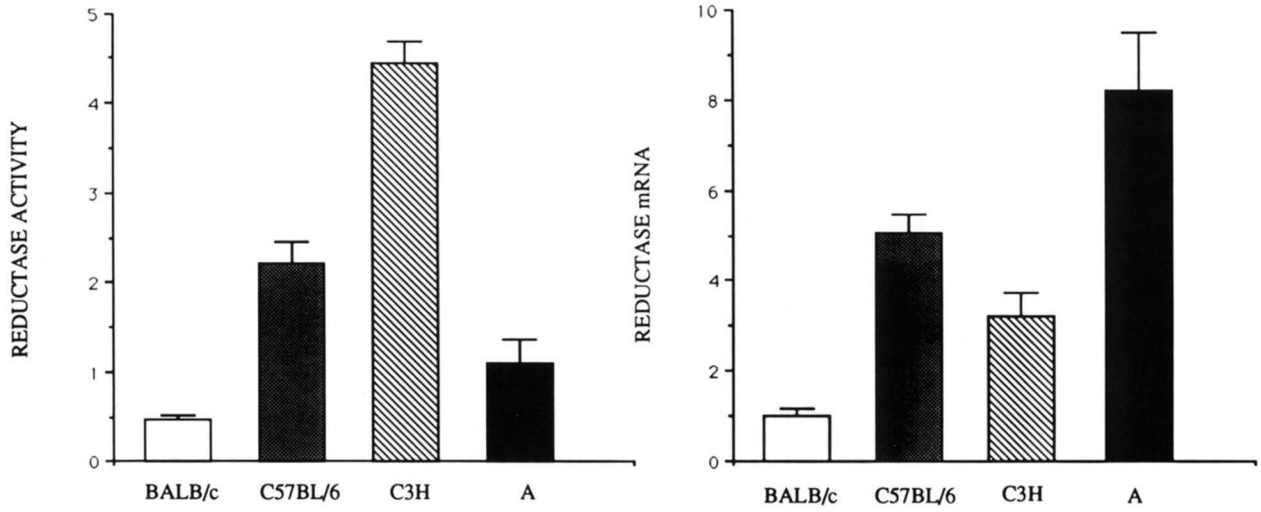

Fig. 2. Levels of hepatic HMG-CoA reductase activity and mRNA among four strains of mice. HMG-CoA reductase activities are expressed as nmol mevalonic acid formed per min per mg protein ± the standard error given in Table 1. The mRNA levels represent the relative densitometric units obtained by the scanning of Northern blots ± the standard error. The number of animals examined for mRNA levels was five for BALB/cJ and C57BL/6J, four for C3H/HeJ, and three for A/J.

profiles and susceptibility to the early stages of atherosclerosis (30).

Diurnal cycle

To test whether the diurnal cycle contributes in part to the genetic variation in reductase expression, perhaps by altering the phasing of the cycle, we determined reductase activity throughout the day. Strains BALB/c and C57BL/6 were found to exhibit very similar periodicities (**Fig. 3**).

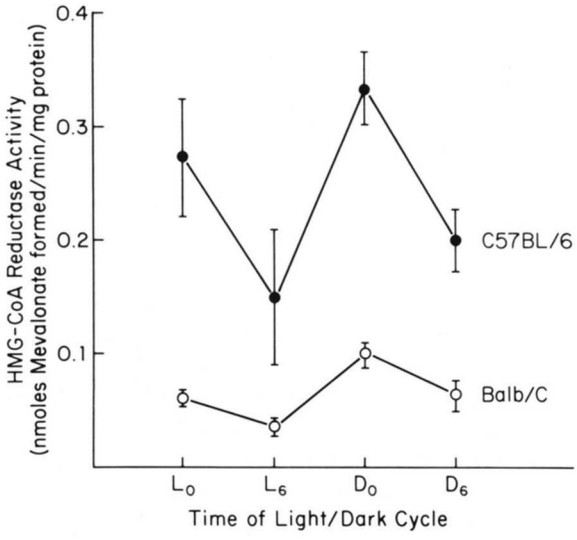

Fig. 3. Diurnal cycle of hepatic HMG-CoA reductase specific activity in mouse strains C57BL/6 and BALB/c. Liver microsomes were prepared at the designated times. LO, the onset of light cycle; L6, the middle of light cycle; DO, the onset of dark cycle; D6, the middle of dark cycle.

For both strains, reductase activity was lowest at the midpoint of the 12-h light cycle and highest at the beginning of the dark cycle. This differs somewhat from the diurnal pattern previously observed in the rat where enzyme activity is maximal at the midpoint of the dark cycle (15, 31). The hepatic reductase activity of strain C57BL/6 was 3- to 5-fold higher than that of the strain BALB/c throughout the 24-h diurnal cycle, indicating that the activity variation is largely independent of the cycle.

Tissue-specific expression

To determine whether the variation is systemic, affecting reductase gene expression in all tissues, or whether it acts in a tissue specific manner, we determined the tissue distribution of enzyme activity for strains BALB/c and C57BL/6 after isolating microsomes from liver, spleen, brain, and heart. While the hepatic enzyme activity of C57BL/6 mice was higher than that of BALB/c mice, other tissues exhibited similar enzyme activities (**Table 2**). Thus, the mutation affecting reductase expression exhibits tissue-specific expression. It is noteworthy that extrahepatic tissues exhibit relatively high HMG-CoA reductase specific activities. This suggests that the overall contribution of extrahepatic tissues to sterol synthesis may be substantial. A major contribution of extrahepatic tissues to sterol synthesis has been observed in certain other mammals (32).

Inheritance pattern

The inheritance of hepatic reductase expression was studied using the CXB recombinant inbred (RI) strains derived from the progenitor strains BALB/cBy (designated "C") and C57BL/6By (designated "B") (33). The

TABLE 2. Tissue distribution of HMG-CoA reductase specific activity

Strains	Tissue			
	Liver	Spleen	Brain	Heart
BALB/c	0.06 ± 0.01 (6)	0.04 ± 0.01 (8)	0.12 ± 0.03 (4)	0.04 ± 0.05 (6)
C57BL/6	0.29 ± 0.10 (6)	0.05 ± 0.02 (3)	0.11 ± 0.03 (4)	0.05 ± 0.02 (3)

Values are expressed as nmol mevalonic acid produced/min per mg protein ± the standard error. The number of animals examined is indicated in parentheses.

seven strains were derived by independent inbreeding beginning with the F_2 generation and are designated (CXB)D, -E, -G, -H, -I, -J and -K. Each RI strain consists of a unique combination of genes derived from the parental strains. The strains have been typed for numerous genetic markers, including a number of genes involved in cholesterol metabolism (17, 18, 28, 29, 34). Since linked genes will tend to cosegregate among the set of RI strains, the set can be used to map genes by comparisons of genetic variations with the stain distribution patterns of previously typed genetic markers. They can also be used to examine physiological effects of genetic variations, as discussed below.

The levels of hepatic reductase specific activities among the (CXB) RI set are shown in **Fig. 4**. Strains (CXB)E, -G, -H, -J, and -K exhibited specific activities similar to the low parent, BALB/c, while strains (CXB)D and -I exhibited activities similar to the high parent, C57BL/6. This pattern of inheritance, in which the RI strains resemble one parent or the other, suggests that between these parental strains reductase expression is determined by a single major locus. If reductase expression were determined by two or more unlinked major loci, some of the RI strains would be expected to exhibit nonparental phenotypes since new combinations of the loci would result from independent segregation. It is noteworthy, however, that the reductase activities of the RI strains differ slightly from the parental strains. The RI strains resembling BALB/c all exhibit somewhat higher activity than the BALB/c parent. Although the activities of strains (CXB)G, -H, -J and -K were not significantly different from BALB/c, the activity of the (CXB)E strain was significantly greater than BALB/c. The most likely explanation for this is that one or more minor loci differing between the parental strains also affect reductase expression; given the multiple levels at which reductase is regulated and the large variation observed among different inbred strains (Table 1), this possibility is not surprising.

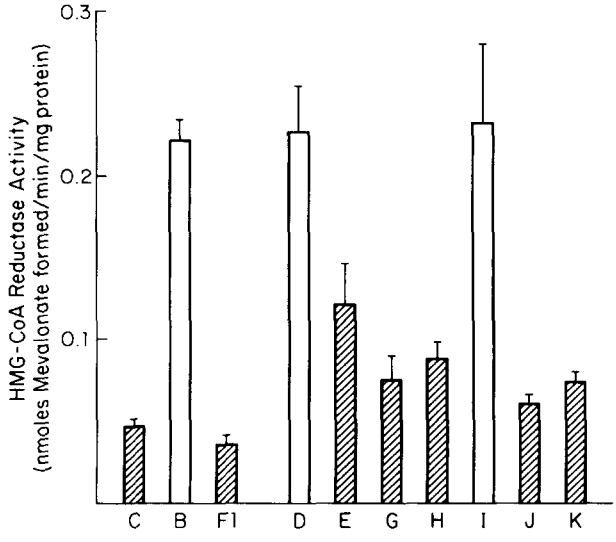

Fig. 4. Hepatic HMG-CoA reductase specific activity in BALB/c, C57BL/6, F1, and the (CXB) recombinant inbred strains derived from the parental strains. Open bars are strains with reductase activity similar to BALB/c; hatched bars are strains with reductase activity similar to C57BL/6. All mice were maintained on chow diets.

Inheritance of reductase expression was also examined in (BALB/c × C57BL/6)F1 progeny. Interestingly, the F1 animals exhibited a phenotype resembling the BALB/c parent (Fig. 4). Such a recessive/dominant mode of inheritance suggests that the mutation does not correspond to a *cis*-acting function of the HMG-CoA reductase structural gene locus, such as a change of the reductase promoter affecting transcription or a change in the reductase transcript affecting RNA stability, since such *cis*-acting mutations would be expected to exhibit additive inheritance.

HMG-CoA reductase gene promoter

The *cis*-acting elements responsible for the transcription of the HMG-CoA reductase gene have been extensively studied. In common with several other sterol responsive genes, including those for low density lipoprotein receptor and HMG-CoA synthase, the elements controlling reductase transcriptional regulation in response to sterols are located in the proximal 5' flanking region of the gene. In particular, linker-scanning and deletion mutations have revealed that the principal regulatory element mediating response to sterols resides in a sequence designated the sterol regulatory element-1 (SRE-1) (4).

In order to directly test whether variations that could affect reductase transcription occur between strains C57BL/6 and BALB/c, we determined the sequence of the 5' flanking region from the two strains as well as strains DBA/2

and C58 which represent the extremes of reductase activity (see Table 1). Overall, the 5' flanking sequence showed a high degree of sequence conservation with the hamster HMG-CoA reductase promoter sequence, and the core SRE-1 sequence responsible for mediating sterol responsiveness was perfectly conserved (**Fig. 5**). Among the four strains, the sequences of the 5' flanking region (base -328 to the transcription start site) were identical (data not shown). These results are consistent with the possibility that the activity and mRNA level variations between strains BALB/c and C57BL/6 are determined by *trans*-acting factors affecting HMG-CoA reductase mRNA levels.

Restriction fragment length variants and chromosomal mapping

In an attempt to determine whether the mutations affecting reductase expression occur at the HMG-CoA reductase structural gene locus, we identified restriction fragment length variants of the gene and used these to map the gene by linkage analysis. Altogether, DNA isolated from ten different strains of mice was examined by Southern blotting with a reductase cDNA probe after digestion with 25 different restriction enzymes. Only two enzymes (PstI and PvuII) revealed any variants among these strains. Thus, with the enzyme PstI, strains AKR, C3H, NZB, SWR, and 129 exhibited hybridizing fragments of 5.6, 4.0, 2.8, 2.4, 1.6, and 1.2 kb while strains BALB/c, C57BL/6, C57L, C58, and DBA/2 exhibited fragments of 5.6, 4.0, 2.8, 2.4, 1.2, and 0.6 kb (**Fig. 6A**). With the enzyme PvuII, the former strains exhibited fragments of 6.7, 4.0, 3.4, 3.0, and 0.9 kb while the latter strains exhibited fragments of 6.7, 4.4, 4.0, 3.4, and 3.0 kb (Fig. 6B). Unfortunately, we were unable to identify a restriction fragment polymorphism between strains BALB/c and C57BL/6, and therefore, were unable to test directly whether the reductase structural gene locus segregates with hepatic reductase activity among the strains, as

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MRED    -328                          CTGCAGGTCA AACTCTGAGT TCGGGGTACT CCACCCGCG-

HAMRED        GGATCCCCTA TCGCCCGCGA GGGCGGCGTC CTTGCTGGCG CCCCTCACGG CTCAGGGACC
               *       *  **      *                    *           **     * *
MRED    -289  GCATCCCCTG TTCCCCGCGC GGGCGGCGTC CTTGCAGGCG CCCCTC--GG CTCGGAGACC

HAMRED        AATAAGAAGG TCGTGATGCT GGAACTCGAC CAGCTATTGG TTGGCTCGGC CGTGGTGAGA
                  *       *  *        *  *       * **              *
MRED    -231  AATAGGAAGG CCGCGATGCT GGGACCCGAC CCGTCATTGG TTGGCTCTGC CGTGGTGAGA

                   SRE-1
HAMRED        GATGGTGCGG TGCCCGTTCT CCGCCCGGGT GCGAGCAGTG GGCGGTTGTT AAGGCGACCG
                                                                     *  *
MRED    -171  GATGGTGCGG TGCCCGTTCT CCGCCCGGGT GCGAGCAGTG GGCGGTTGTT AGGGAGACCG

HAMRED        TTCGTGACGT AGGCCGTCAG GCTGAGCAGC CGCCCGGTGA TTGGCTAGGC GATCGGACGA
                                                      *            *
MRED    -111  TTCGTGACGT AGGCCGTCAG GCTGAGCAGC CGCCCGGCGA TTGGCTAGGG GATCGGACGA

HAMRED        TCCTTTCTTA TTGGCGGCCC ---------- -TGAGCGTGC GTAAGCTCAG TTCCTTCCGC
                  *                 ********** **                *      1
MRED    -51   TCCTTCCTTA TTGGCGGCCC GCTGGCGGCC TGGAGCGTGC GTAAGCGCAG TTCCTTCCGC
                                                                      exon 1

HAMRED        CCGAGGCTCC GTTGGCTGGA GACCGCGGCT GGGCCGGCTT TGGGGCCTCC ATTGAGATCC
                 *                    *  *                 *  ***
MRED    +9    CCGGGGCTCC GTTGGCTGGA GACGGCAGCT GGGCCGGCCT ---GGCCTCC ATTGAGATCC

HAMRED        GGAGGTGAGC GGACAGACTC TGGGGACTGG CCGCC
                         * * *   *          *    * **
MRED    +66   GGAGGTGAGC TGGCGGACGC TGGGGACCGG CGGGA
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Fig. 5. Sequence of 5' flanking region of the HMG-CoA reductase gene from mice compared with the 5' flanking region of the hamster gene. Four strains of mice (C57BL/6J, BALB/cJ, DBA/2J, and C3H/HeJ) had identical sequences. Sequences corresponding to the flanking region were amplified by polymerase chain reaction and sequenced in both directions either directly or after subcloning. The mouse sequence is numbered from the first transcription start site for hamster. The hamster promoter sequence ends at −289, and the exon 1 sequence is double underlined. The sequence was aligned with the hamster sequence to yield the best fit. Asterisks indicate a mismatch, and dashes indicate a deletion of one sequence relative to the other. The core sterol regulatory element (SRE-1) sequence is underlined.

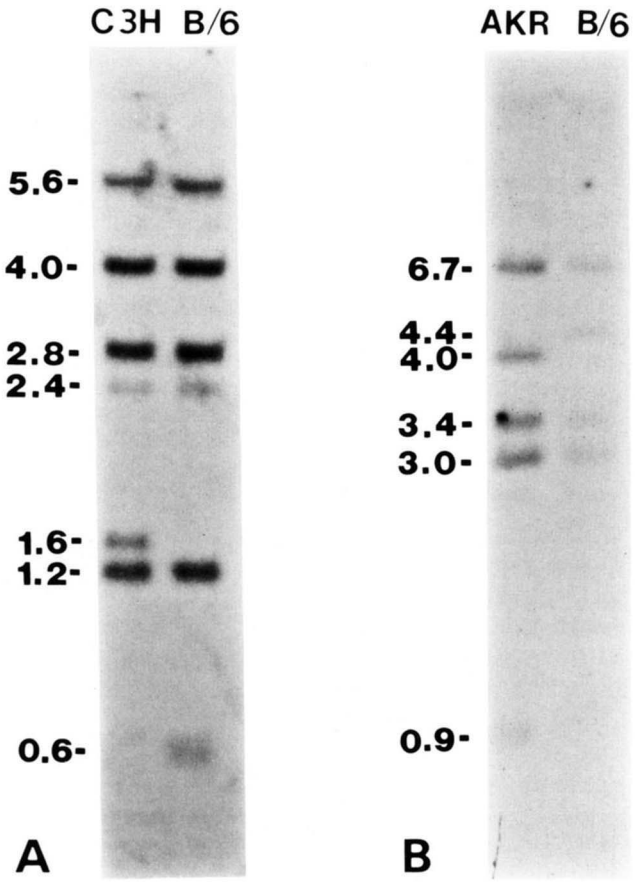

Fig. 6. Restriction fragment length polymorphisms of the HMG-CoA reductase gene among inbred strains of mice. The enzymes PstI (panel A) and PvuII (panel B) revealed polymorphisms that distinguish strains AKR, C3H, NZB, SWR, and 129, from strains BALB/c, C57BL/6, C57L, C58, and DBA/2.

would be expected if mutations of the reductase gene were responsible for the different phenotypes.

The variants were useful, however, in establishing the chromosomal location of the gene and in identifying linked genetic markers through analysis of other sets of strains. Three sets of RI strains, derived from parental strains AKR and C57L (AKXL), C57BL/6 and C3H (BXH), and SWR and C57L (SWXL), differing in the PstI and PvuII restriction patterns were examined (**Table 3**). The results indicated that the HMG-CoA reductase gene has segregated concordantly with the gene for arylsulfatase B (*As-1*) in 34 of 36 RI strains tested. The recombination frequency (r) between these two genes can be estimated from the relationship $r = R/4-6R$) where R is the ratio of recombinants to the total number of strains tested (29). Thus, these results indicate close linkage between the HMG-CoA reductase gene (designated *Hmgcr*) and *As-1*, with an estimated map distance of 1.5 centimorgans (cM). The 95% confidence interval of the map distance (cM) is [0.17, 6.5]. The *As-1* gene has previously been mapped to the distal region of mouse Chromosome 13

TABLE 3. Linkage between HMG-CoA reductase restriction fragment variant and arylsulfatase-B in recombinant inbred strains of mice

Genotype[a]		
As-1	*Hmgcr*	(Progenitor) and RI Strains[b]
a	*a*	(AKR/J), AKXL-5, -6, -8, -14, -17, -21, -24, -37, -38 (SWR/J), SWXL-12, -14, -17 (C3H/HeJ), BXH-3, -8, -9, -10, -11, -19
b	*b*	(C57L/J), AKXL-7, -9, -12, -13, -19, -25, -28, -29 SWXL-4, -15, -16 (C57BL/6J), BXH-4, -6, -7, -12, -14
a	*b*	BXH-2 AKXL-16

[a]The genotype designations *a* and *b* correspond to arylsulfatase B gene (*As-1*) and HMG-CoA reductase gene (*Hmgcr*) allelic types present in the strains. The "a" genotypes are present in parental strains AKR, SWR, and C3H whereas the "b" genotypes are present in strains C57BL/6 and C57L. HMG-CoA reductase alleles were determined using a *PstI* restriction fragment length polymorphism (see text).

[b]Parental strains are in parentheses. AKXL RI strains are derived from AKR/J and C57L/J; SWXL RI strains are derived from SWR/J and C57L/J; and BXH RI strains are derived from C57BL/6J and C3H/HeJ.

near the coat color mutation pearl (*pe*) locus (35). The Chromosome 13 location of the mouse HMG-CoA reductase gene is consistent with previous somatic cell hybrid studies (36; A. Diep, T. Mohandas, A. Lusis, unpublished results) and has been further confirmed by linkage analysis in backcrosses (C. Warden, M. Mehrabian, A. Diep, A. Lusis, unpublished results).

The closely linked *As-1* gene exhibits variation between strains BALB/c and C57BL/6 and has previously been typed among the (CXB) set of RI strains. We, therefore, examined whether this gene segregates with reductase activity phenotype. As shown in **Table 4**, with one exception, RI strain (CXB)J, the strains that have inherited the BALB/c *As-1* allele (designated "C") have low reductase activity while the strains that have inherited the C57BL/6 *As-1* allele (designated "B") have high reductase activity. This apparent cosegregation between the reductase gene and enzyme activity in this small number of strains is not significant ($P > 0.05$). Based on the distance between the

TABLE 4. Association between HMG-CoA reductase activity and arylsulfatase-B genotypes in (CXB) strains

As-1 Genotype[a]	Reductase Activity[a]	RI Strains
a	*L*	(CXB)K, (CXB)H, (CXB)E, (CXB)G, BALB/c
b	*H*	(CXB)I, (CXB)D, C57BL/6
b	*L*	(CXB)J

[a]The designations *a* and *b* correspond to arylsulfatase B (*As-1*) genotypes. The designations *L* and *H* represent the hepatic HMG-CoA reductase low and high specific activity phenotypes.

As-1 locus and the HMG-CoA reductase gene locus, 1.5 cM, the discordancy of the two loci among RI strains is expected to be about 6%, since as compared to backcrosses, recombination is amplified by a factor of about 4 among RI sets due to multiple opportunities for recombination during construction of the strains. This compares with the observed frequency of recombination of 14% (1 out of 7) between the *As-1* gene and the reductase activity phenotype.

Genetic control of dietary response

The above results, particularly the recessive/dominant mode of inheritance of the activity variation, suggest that a mutation distinct from the HMG-CoA reductase structural gene and affecting reductase transcription or mRNA turnover is responsible for the difference in hepatic enzyme expression between strains BALB/c and C57BL/6. To test whether the mutation controls the responsiveness of the mice to diet, we maintained the mice for 3 weeks on a high-fat diet containing 7.5% cocoa butter, 1.25% cholesterol, and 0.5% cholic acid. This diet has been widely used to study susceptibility of mice to the development of aortic fatty streaks (17). As shown in **Fig. 7**, the two strains respond very differently to the dietary challenge. Hepatic reductase activity was only slightly depressed (1.6-fold) in strain BALB/c while it was decreased more than 13-fold in strain C57BL/6. Thus, whereas the hepatic reductase specific activity of strain C57BL/6 on the chow diet was nearly 5-fold higher than strain BALB/c, on the high-fat diet the reductase activity of C57BL/6 was similar to or less than that of BALB/c.

This striking difference in dietary response was accounted for entirely by changes in mRNA levels. Hepatic HMG-CoA reductase mRNA levels were essentially unaffected by the high-fat diet in BALB/c mice while they decreased in proportion to activity (about 13-fold) in C57BL/6 mice (Fig. 7).

The data of Packie and Kandutsch (16), replotted in Fig. 7, show that sterol synthesis parallels HMG-CoA reductase expression in the two strains on a chow diet. Thus, as in tissue culture cells, the enzyme appears to be rate-limiting for sterol synthesis.

The hepatic reductase activities of the seven (CXB) RI strains when maintained on the high-fat diet are shown in **Table 5**. All of the strains exhibited similar activities, ranging from 0.021 to 0.030 nmol mevalonic acid formed per min per mg microsomal protein. Thus, when challenged with a high cholesterol diet, the expression of the reductase genes in both strains (as well as all of the RI strains) is depressed to roughly the same basal level. This suggests that the difference in enzyme expression on a low-fat (chow) diet may represent a difference in either the feedback pathway regulating reductase expression in response to cellular sterol levels or in the sterol levels themselves.

Cellular and plasma lipid levels

As discussed above, the difference in reductase expression between BALB/c and C57BL/6 mice on the chow diet could be explained by a difference in hepatic cholesterol or possibly triglyceride levels. However, as shown in **Table 6**, the strains had similar hepatic cholesterol levels

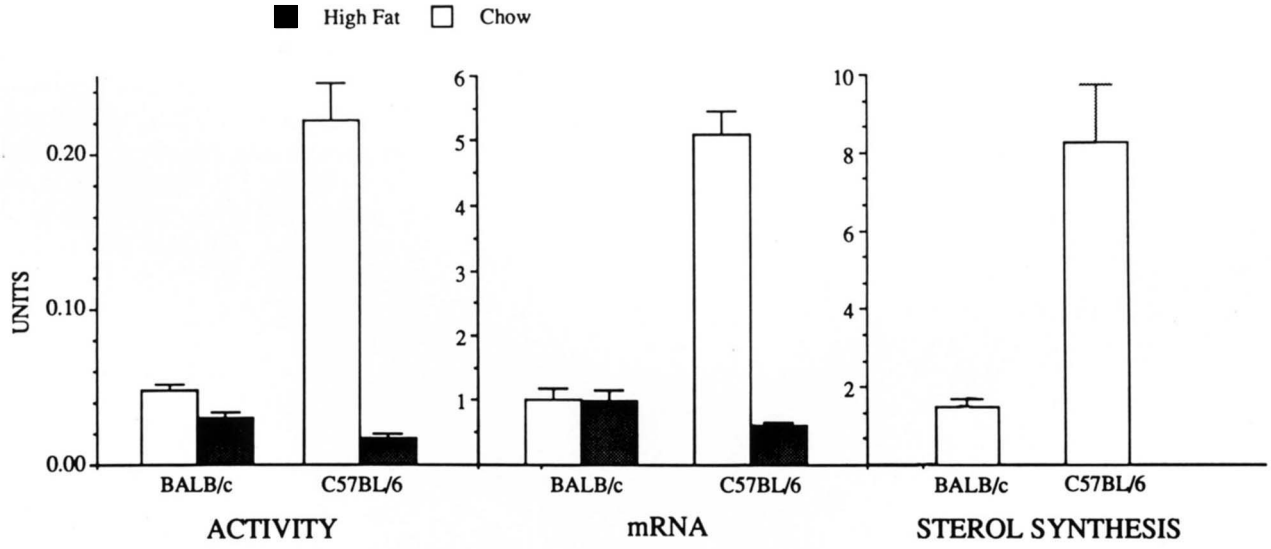

Fig. 7. Coordinate genetic control of hepatic HMG-CoA reductase specific activity, HMG-CoA reductase mRNA levels, and sterol synthesis. C57BL/6 and BALB/c mice were maintained on either chow (open bars) or high fat (shaded bars) diets for 3 weeks, when hepatic HMG-CoA reductase activity and relative mRNA levels were determined. Also plotted for a comparison are the rates of sterol synthesis in the two strains on a chow diet as determined by Packie and Kandutsch (16) on a chow diet. Rates of sterol synthesis are expressed as nmol [1-^{14}C]acetate converted to sterols per g liver per hour.

TABLE 5. Levels of hepatic HMG-CoA reductase specific activity and mRNA in mice maintained on chow, high fat, or cholestyramine+ mevinolin diets

Strain	Diet		
	High Fat	Normal Chow	Cholestyramine + Mevinolin
BALB/c activity[a]	0.030 ± 0.004 (4)	0.048 ± 0.004 (20)	16.02 ± 3.98 (5)
BALB/c mRNA[b]	0.99 ± 0.16 (4)	1.00 ± 0.17 (5)	13.5 ± 0.3 (3)
C57BL/6 activity	0.017 ± 0.003 (5)	0.222 ± 0.024 (10)	24.02 ± 1.23 (5)
C57BL/6 mRNA	0.60 ± 0.05 (4)	5.08 ± 0.37 (5)	76.8 ± 3.9 (5)
(CXB)D activity	0.027 ± 0.004 (3)	0.226 ± 0.028 (4)	17.55 (2)
(CXB)E activity	0.028 ± 0.002 (3)	0.122 ± 0.026 (9)	19.79 (2)
(CXB)G activity	0.021 ± 0.004 (3)	0.076 ± 0.014 (4)	14.33 ± .95 (3)
(CXB)H activity	0.021 ± 0.004 (3)	0.088 ± 0.010 (11)	18.10 ± 4.39 (3)
(CXB)I activity	0.025 ± 0.004 (3)	0.232 ± 0.048 (13)	15.46 ± 6.65 (3)
(CXB)J activity	0.030 (2)	0.060 ± 0.006 (4)	16.48 ± 5.70 (3)
(CXB)K activity	0.026 ± 0.001 (3)	0.074 ± 0.005 (9)	19.30 ± 3.60 (3)

[a]Values are expressed as nmol mevalonic acid formed/min per mg protein ± standard error with number of animals tested in parentheses.
[b]mRNA values represent relative intensities observed after densitometric scanning of Northern blots ± standard error with number of animals tested in parentheses. All values were normalized against signals obtained for β-tubulin mRNA.

when maintained on either the chow or high fat diets. It is noteworthy that BALB/c hepatic reductase was almost totally unresponsive to the high fat diet despite an approximately 10-fold increase in hepatic cholesterol levels. Plasma total cholesterol and triglyceride levels differed only slightly between the strains (Table 6). The major difference in lipid levels was a greater decrease in HDL cholesterol levels on the high fat diet in strain C57BL/6 as compared to BALB/c (Table 6). The HDL cholesterol levels, however, segregated independently of HMG-CoA reductase activity, indicating that separate genes are involved (**Table 7**).

Response to cholestyramine-mevinolin treatment

The combination of cholestyramine (a chelator of bile acids that interrupts the enterohepatic circulation) and mevinolin (a strong competitive inhibitor of HMG-CoA reductase) has previously been shown to result in a large induction of reductase expression in experimental animals (15). When BALB/c and C57BL/6 mice were fed the drug combination for 4 days, the levels of hepatic HMG-CoA reductase and mRNA increased dramatically (Table 5). Interestingly, the increase in reductase activity levels in both strains was much larger than could be accounted for by the increase in reductase mRNA levels, presumably reflecting additional regulation at the level of translation and enzyme turnover (Table 5). For example, in strain BALB/c, hepatic reductase specific activity increased about 330-fold in response to the drugs, whereas reductase mRNA levels increased only about 13-fold. In strain C57BL/6, hepatic reductase activity increased about 110-fold, whereas reductase mRNA levels increased only

TABLE 6. Plasma and hepatic lipid levels of inbred mouse strains maintained on chow or high fat diets

Strain	Diet	Plasma Lipids[a]			Hepatic Lipids[b]	
		Total Cholesterol	HDL Cholesterol[c]	Total Triglycerides	Total Cholesterol	Total Triglycerides
		mg/dl			*ng lipids/mg protein*	
BALB/c	Chow	71.0 ± 9.0	50.9 ± 5.8	26.4 ± 13.3	13.6 ± 0.6	24.1 ± 20.0
BALB/c	High fat	151 ± 24.5	35.8 ± 10.6	8.8 ± 2.0	140.0 ± 3.2	54.0 ± 10.0
C57BL/6	Chow	76.9 ± 15.4	48.5 ± 5.2	7.3 ± 2.3	10.2 ± 2.8	42.7 ± 11.0
C57BL/6	High fat	115 ± 17.8	21.7 ± 3.8	10.2 ± 3.2	169.0 ± 31.0	63.5 ± 3.0

[a]Values are expressed as mg/dl (mean ± SD); 10–15 animals were examined in each group.
[b]Values are expressed as ng lipids/mg protein (mean ± SD); three animals were examined in each group.
[c]High density lipoprotein cholesterol was determined after precipitation of LDL/VLDL lipoproteins using heparin as described in Methods.

TABLE 7. Segregation patterns of HMG-CoA reductase activity phenotypes and candidate loci among CXB recombinant inbred strains

Locus	Chromosome	CXB Strains							Reference
		D	E	G	H	I	J	K	
HMG-CoA reductase activity	?	B[a]	C	C	C	B	C	C	this report
HDL cholesterol levels	1	C	B	C	C	B	C	C	17
Apoa2	1	C	B	C	B	B	C	C	18, 57
Svp-2 (linked to *Apoe*)	7	C	C	C	C	C	B	B	18, 57
Apoa1	9	C	C	C	B	B	B	C	18, 57
Apob	12	B	C	C	C	C	B	C	18, 57
As-1 (linked to *Hmgcr*)	13	B	C	C	C	B	B	C	35
Ea-4 (linked by *Osbp*)	19	C	B	B	C	B	B	B	57, 58

[a]The designations "B" and "C" correspond, respectively, to the C57BL/6 and BALB/c phenotypes or genotypes.

about 15-fold (Table 5). This is in contrast to the results with the high-fat diet, where reductase activity and mRNA levels were similarly altered (Table 5). As in the case of the high-fat diet, both strains achieved similar maximal levels of hepatic reductase activity after cholestyramine–mevinolin treatment (Table 5). However, the drug treatment resulted in a marked difference in reductase mRNA levels. The difference was very similar to that observed under conditions of chow feeding, with strain C57BL/6 exhibiting about 5-fold higher levels than BALB/c (Table 5). These results suggest that posttranscriptional regulation of reductase expression may predominate at higher levels of cellular sterols. When maintained on the cholestyramine–mevinolin diet, all of the (CXB) RI strains exhibited similar levels of hepatic reductase specific activity, ranging from 14.3 to 19.8 nmol mevalonate formed per min per mg microsomal protein (Table 5). It is curious that on the cholestyramine–mevinolin diet strain C57BL/6 mice had higher levels of reductase mRNA than all of the RI strains (Table 5). The explanation is unknown, although it presumably reflects unknown genetic or environmental influences.

Effect of genetic variation on other sterol responsive genes

The above results suggest that the variation between strains BALB/c and C57BL/6 affects HMG-CoA reductase regulation in *trans* (see Discussion). Since HMG-CoA reductase exhibits coordinate regulation with a number of other sterol responsive genes, it is of interest to examine whether expression of these genes, which include HMG-CoA synthase and the low density lipoprotein receptor, is also affected by the genetic variation. Preliminary results suggest that this may be the case. Thus, on a chow diet, the levels of hepatic HMG-CoA synthase mRNA in C57BL/cJ mice were 180 ± 4% (mean ± SD) of those in BALB/cJ mice, and in response to a high-fat diet (as in Table 5) synthase mRNA levels decreased an average of 7.7-fold in C57BL/6 mice but only 4.2-fold in BALB/c mice. For these experiments the quantitation was performed by Northern blotting using a rat HMG-CoA synthase cDNA probe (37) as well as a tubulin control probe essentially as described in Fig. 1 for HMG-CoA reductase. Another gene that is responsive to sterols and also to bile acids, is cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis (38). Recent studies have revealed that the response of cholesterol 7α-hydroxylase to a high-fat atherogenic diet containing cholic acid in strains BALB/c and C57BL/6 parallels the response observed for HMG-CoA reductase. Thus, both at the level of enzyme activity and mRNA, strain C57BL/6 was highly responsive to the diet while strain BALB/c was relatively unresponsive (39).

DISCUSSION

A mouse model for the genetic control of cholesterol homeostasis

Studies of mammalian cells in culture as well as of animals have shown that cholesterol homeostasis is maintained by feedback regulation of both the de novo synthesis of cholesterol and the cellular uptake of cholesterol (14). The importance of cholesterol uptake, mediated largely by the low density lipoprotein receptor, has been clarified by the analysis of mutations affecting receptor function that occur in familial hypercholesterolemia, and detailed studies of the biochemical effects of receptor mutations were made possible by the characterization of a rabbit animal model for the disease (14). In contrast, genetic studies of cholesterol synthesis have been restricted largely to cultured cells. Such cell culture studies have clarified mechanisms involved in the regulation of sterol synthesis and have revealed that the enzyme exhibits complex feedback regulation at several levels, including transcription, translation, and enzyme turnover

(2, 6, 11, 40, 41). Genetic studies, particularly of CHO mutant cell lines that fail to down-regulate sterol synthesis in the presence of 25-hydroxycholesterol, have also been informative (7, 10, 42–44). Some of these mutations have been shown to coordinately affect the expression of several enzymes of the sterol biosynthetic pathway as well as the low density lipoprotein receptor. Certain mutant cells appear to have functionally lost a common factor that mediates the effects of sterols on the sterol regulatory element-1 (SRE-1) (45, 46). The identity of the genes involved, however, is unknown, although one 25-hydroxycholesterol-resistant mutant was found to be a fatty acid auxotroph (47). Whether similar genetic variations occur in nature is unclear. As yet, little is known of genetic influences that affect sterol synthesis in humans or other mammals. Nor is there presently an understanding of the possible physiological consequences of such influences, although it has been suggested that variations of hepatic HMG-CoA reductase may contribute to certain hyperlipidemias and to gallstone disease (48).

Because of the difficulty of directly examining the multiple genetic and environmental factors that control cholesterol metabolism in humans, we undertook this study in mice. The mouse has important advantages for genetic analysis, including the availability of numerous inbred strains, each representing a unique gene pool, as well as recombinant inbred strains, which simplify gene mapping and analysis of complex genetic traits (29). Although mice differ from humans in the relative amounts of various circulating lipoproteins, there are many counterparts between cholesterol biosynthesis and metabolism in rodents and humans (34). Thus, the genetic control of cholesterol homeostasis in mice should clarify aspects of mammalian cholesterol regulation in general. The original studies of Packie and Kandutsch (16) established that inbred strains of mice exhibit remarkable variations in the levels of hepatic HMG-CoA reductase as well as sterol synthesis. Our present results have confirmed these findings, and our larger survey indicates that inbred strains differ in some cases by more than 20-fold in hepatic HMG-CoA reductase levels. This unusually high degree of polymorphism is unexpected, especially for an enzyme that plays such a key role in metabolism. Whether a similar level of polymorphism occurs among other mammals, including humans, is unclear. In any case, the genetic variations should permit studies of the genetic and molecular mechanisms governing reductase expression and sterol synthesis in an animal model. The studies described in this report have focused on only one variation, occurring between strains BALB/c and C57BL/6, which acts at the level of reductase mRNA. Variations that affect other important aspects of reductase expression, such as translational or posttranslational control, also appear to occur among inbred strains. Our results provide a striking example, at the molecular level, of the interaction of dietary and genetic factors in cholesterol homeostasis, and of the physiological consequences of such interactions.

Molecular basis of activity variations

The hepatic HMG-CoA reductase activity variation between strains BALB/c and C57BL/6 was studied in detail. Strain C57BL/6 showed 4- to 5-fold higher hepatic HMG-CoA reductase activity than strain BALB/c. The activity variation could be explained entirely (within experimental error) by differences in reductase mRNA; that is, the strains differed by a factor of 4–5 in both reductase activity and reductase mRNA levels. This variation is tissue-specific and is not due to a phase shift of the reductase diurnal cycle. The BALB/c and C57BL/6 strains did not differ in the levels of hepatic cholesterol or triglycerides on chow and high-fat diets. Thus, it appears unlikely that levels of cellular sterols are responsible for the reductase variation, although it is possible that the effects could be explained by differences in cellular sterol compartmentalization or differences in the levels of nonsterols or oxysterol metabolites involved in reductase feedback regulation. In genetic crosses the reductase phenotypes segregated as a single major Mendelian gene, although we cannot formally rule out an interacting two-locus model for the control of reductase expression. Analysis of the F1 cross between BALB/c and C57BL/6 showed low activity, similar to the BALB/c parent. Such a pattern of recessive inheritance suggests that the genetic variation controls reductase expression in *trans* since, as discussed above, mutations of the reductase structural gene locus would generally be expected to result in additive inheritance. Consistent with this conclusion is the finding that the sequence of the reductase gene promoter is identical in the two strains, although other, as yet unknown, promoter or enhancer elements in the reductase gene could affect transcription. Moreover, the data suggesting that the mutation has effects on other sterol responsive genes (HMG-CoA synthase and cholesterol 7α-hydroxylase) argues against a *cis*-acting mutation of the HMG-CoA reductase gene. Thus, we conclude that the mutation probably affects a *trans*-acting factor that controls the transcription of the reductase gene or the turnover of reductase mRNA.

In an effort to provide definitive evidence as to whether or not mutations of the reductase gene locus are responsible for the activity phenotypes, we attempted to determine whether the reductase gene cosegregates with the phenotypes. Since we were unable to identify restriction fragment length variants of the reductase gene between BALB/c and C57BL/6, we examined the segregation pattern of the closely linked arylsulfatase B (*As-1*) gene locus (see below). The results showed that the *As-1* gene fails to cosegregate with the reductase activity phenotypes in one of seven RI strains ($P > 0.05$), consistent with the possibility that mutations of the reductase gene locus are not responsible. Although several proteins that interact with

the reductase promoter have been identified (49), the transcriptional control of reductase gene expression is still poorly understood, and the factors responsible for feedback repression are as yet unknown. Likewise, little is known of factors that control reductase mRNA turnover, although recent studies with rats have shown that glucocorticoids and thyroid hormone can regulate reductase mRNA stability (9). Therefore, identification of the gene product involved in the C57BL/6–BALB/c genetic variation may prove difficult. This is clearly of importance, however, since it would clarify the function of a known factor or reveal a new one. A number of potential candidate genes that could affect HMG-CoA reductase expression can be eliminated on the basis of their chromosomal location (see below).

Chromosomal organization

Using DNA polymorphisms, we mapped the gene for HMG-CoA reductase gene (*Hmgcr*) to the distal region of mouse Chromosome 13, tightly linked to the gene for arylsulfatase B (*As-1*). The close linkage between the HMG-CoA reductase gene and the arylsulfatase B gene has been conserved in humans, since the human homologs of both genes map to the proximal region of the long arm of Chromosome 5 (50, 51). The genes for human HMG-CoA reductase and HMG-CoA synthase are both located in human Chromosome 5 (band 5p13 and 5q13.3-q14, respectively) (37). This linkage is conserved in the mouse, where the HMG-CoA reductase gene is about 20 cM distal to the HMG-CoA synthase gene, although there is a rearrangement with respect to the position of the centromere, which in the mouse does not interrupt the linkage of the genes (M. Mehrabian, C. Warden, A. Diep, A. Lusis, unpublished results). Another sterol-responsive gene, the low density lipoprotein receptor gene, is unlinked to the reductase or synthase genes in both humans (where it resides on Chromosome 19) and mice (Chromosome 9) (52).

As discussed above, our results suggest that the reductase activity variation is not due to polymorphisms of the HMG-CoA reductase structural locus. A number of other potential candidate genes for the activity variation can also be eliminated on the basis of their segregation patterns among the CXB RI set (Table 7). These include the genes for apolipoprotein A-II (*Apoa2*), apolipoprotein A-I (*Apoa1*), and apolipoprotein B (*Apob*). Certain other genes which have not been typed among the CXB RI set are also unlikely to be responsible for the activity variation based on the segregation patterns of linked markers. These include apolipoprotein E (*Apoe*) which is tightly linked to the *Svp-2* marker on mouse Chromosome 7, and the oxysterol binding protein gene (*Osbp*) which is linked to the *Ea-4* marker on Chromosome 19 (Table 7).

Physiological consequences

One of the surprising conclusions to emerge from these studies is that relatively large variations in hepatic reductase activity and in sterol synthesis need not have large effects on blood cholesterol levels. The explanation for this is still unclear, but it may result in part from extrahepatic sterol synthesis (Table 2). Direct measurements of the relative contribution of various tissues to total body sterol synthesis have shown that extrahepatic synthesis is substantial in certain species (32). It should also be noted that peroxisomes contain many biosynthetic enzymes for cholesterol synthesis and may well contribute to cholesterol homeostasis (53). Recent studies indicate that strains BALB/c and C57BL/6 do not exhibit significant differences in cholesterol absorption on a chow diet (S. Dueland, D. Machleder, A. Lusis, R. Davis, unpublished results), indicating that the differences in reductase expression do not result from differences in cholesterol uptake. On the other hand, the strains may well differ in rates of cholesterol catabolism, since strain C57BL/6J mice have elevated cholesterol 7α-hydroxylase activity on a chow diet relative to BALB/c mice (39).

Genetic–environmental interactions

The genetic variations between BALB/c and C57BL/6 have a remarkable effect on the response of HMG-CoA reductase to diet. Whereas strain C57BL/6 exhibited striking repression of hepatic reductase activity and mRNA levels in response to a high-fat diet, strain BALB/c was almost entirely resistant. Thus, it appears that strain C57BL/6 mice have a more sensitive feedback mechanism regulating reductase expression in response to sterols than do BALB/c mice.

Certain studies suggest that similar genetic variations occur in human populations. For instance, the Masai, a Nilo-Hamitic tribe of East Africa, have extremely high fat intake but appear to maintain relatively low blood cholesterol levels by means of unusually efficient negative feedback regulation of cholesterol synthesis (54). On the other hand, Arctic Eskimos appear to have relatively poor repression of cholesterol synthesis in response to dietary cholesterol, and their serum cholesterol levels have been found to be proportional to their daily cholesterol intake (55). Similar differences in response to dietary cholesterol have been identified in monkeys (15).

Recently, Berg (56) identified genes that control environmental responsiveness, so-called "variability genes," using twin studies. Thus, certain alleles of the apolipoprotein B and cholesteryl ester transfer protein genes were associated with significantly greater variability of cholesterol phenotypes than other alleles. Presumably, this is due to the fact that certain alleles of these genes are more sensitive to environmental influences than others, resulting in larger variability between the identical twins. The

molecular basis of such genetically determined variability is unknown. The example of HMG-CoA reductase in mice now provides an example of how such environmental variability is influenced by genetics. As part of our preliminary work, we found that when mice were maintained several animals to a cage, the hepatic reductase variation was quite large. This was particularly true for strain C57BL/6, where the coefficient of variation for reductase specific activity approached 50%, while in strain BALB/c the coefficient of variation was about only 30%. When mice were housed individually, the coefficient of variation of strain C57BL/6 mice was decreased dramatically, to about 30%, whereas that for strain BALB/c mice remained about 30%. These findings can now be explained by the greater responsiveness of C57BL/6 mice to dietary or other environmental influences.

It is interesting that the strains BALB/c and C57BL/6 exhibited very similar levels of HMG-CoA reductase expression at the extremes of cellular sterol levels (high-fat diet or cholestryamine-mevinolin diet) but differed markedly on a chow diet. This suggests that the genetic alteration between the strains determines the concentration of cellular sterols required for feedback regulation of enzyme expression; at sterol levels produced by a chow diet, the enzyme in strain BALB/c is maximally repressed whereas that in C57BL/6 is not.

Conclusions

The large variations in hepatic cholesterol synthesis rates between different species and within individuals of one species presumably reflect net sterol balance across the liver. This is a complex process involving cholesterol absorption and synthesis, bile acid absorption, synthesis, and degradation, and plasma lipoprotein secretion and uptake. The genetic alterations in hepatic sterol synthesis described here may act at a variety of levels and need not directly alter gene expression in the liver. In the case of the genetic variation between strains BALB/c and C57BL/6 described in this study, it is clear that differences in cholesterol absorption are not involved, but the observed differences in hepatic sterol synthesis could, nevertheless, result from other extrahepatic events (for example, alterations in bile acid absorption or degradation) as well as hepatic events.

It is remarkable that large variations in the expression of HMG-CoA reductase, which appears to be a sensitive barometer of sterol balance, are not associated with substantial alterations of plasma cholesterol levels among the various inbred strains of mice examined. Since increased hepatic cholesterol synthesis would increase cholesterol flux through plasma, we assume that there are homeostatic mechanisms or compensatory genetic variations that maintain the relatively similar plasma cholesterol levels. It seems likely that large interindividual variations in cholesterol synthesis also occur in humans and other mammals, but, if so, the impact of such variations on plasma lipoproteins is unclear.

Perhaps the most interesting finding to emerge from this work is the striking influence of genetic factors on the dietary responsiveness of HMG-CoA reductase expression and sterol synthesis. Some of the diets used in our studies were rather "unnatural", but it is likely that they approximate the extremes of reductase repression or induction. It will be of interest to examine the effects of more subtle dietary alterations, and of various classes of lipids, including cholesterol, fatty acids, and bile acids, on reductase expression in various genetic backgrounds.

Finally, it is clear from these and other studies that many important questions remain in our understanding of sterol metabolism. Reductionistic approaches have elucidated mechanisms that are fundamental to cholesterol homeostasis, and studies of cells in culture have been particularly informative. But, given the many complex interactions governing cholesterol balance, it seems that less reductionist approaches will also be necessary for an overall understanding of the system.

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