vacuole contents after mating, since some of these molecules may be involved in vacuole movement during cell division. We have tested the following mutants for a defect in vacuole mixing in zygotes: *kar1*-1, which is defective in nuclear fusion (*6*), a *pep4* strain, defective in some vacuole proteases (*7*), two *tub2* strains, defective in β-tubulin (*8*), and two *act1* strains, defective in actin (*9*). The matings were performed with the defect present in both mating types. In all cases, dye transfer was normal at both the permissive and, where applicable, restrictive temperatures. Since these mutations may not completely abolish protein function, our studies do not rule out important roles for these proteins in vacuole exchange.

The observation that parental vacuoles never fuse within the zygote, yet mix their contents, contrasts with the behavior of other yeast organelles. During mating, the two parental nuclei fuse, mitosis occurs, and the nucleus divides. One nucleus remains with the zygote and the other nucleus is directed into the bud (*10*). Fusion of the parental mitochondria is rare, and uniparental contribution of mitochondria to the bud often occurs (*11–14*).

It seems likely that intervacuolar dye transfer is mediated by vesicular traffic. In mammalian cells, vesicle-mediated traffic occurs both in endocytosis (*15, 16*) and in biosynthetic transport (*17*). Vesicle traffic between homologous organelles has been well documented in mammalian Golgi (*18*). The Golgi do not fuse during inter-Golgi traffic in vitro. Instead, proteins are moved in a vesicle-mediated process (*19*). It has recently been reported that mammalian lysosomes equilibrate their contents after syncytia formation (*20*). The mechanism by which this occurs is as yet unknown. Possibly, the equilibration observed in mammalian lysosomes is analogous to yeast vacuole behavior during mating.

The observations of intervacuole and interlysosome exchange define a new pathway in interorganelle traffic. Further studies of intervacuole exchange are needed to determine why the transfer is rapid and why it does not happen immediately after cytoplasmic mixing. In addition, our observations demonstrate that vacuole contents can be transferred both from parental vacuoles into the bud and from the bud to the parental vacuoles. Perhaps the transfer is triggered by a cytoskeletal rearrangement. Observation of the trails connecting each parental vacuole to the bud suggests that the bud vacuole may form by directed vesicular traffic from the parental vacuoles to a specific vacuole site in the bud. This model is further supported by the observation that the *ade2* dye appears first in the bud and then in the *ADE* parental vacuole. The vesicle movement between the parental and bud vacuoles may continue in both directions until cytokinesis occurs (or until vacuole formation is complete). Thus, the phenomenon observed in yeast zygotes may provide insight into both vacuole and lysosome division and segregation.

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22. We thank B. Guthrie, M. Grunstein, L. Hicke, and G. Payne for helpful discussions, and D. Botstein for providing DBY1398 [*MATa ade2 ura3*]. Supported by a grant from the National Institutes of Health. L.S.W. was supported by an NIH National Research Service award.

3 March 1988; accepted 2 June 1988

Low Plasma Cholesterol Levels Caused by a Short Deletion in the Apolipoprotein B Gene

STEPHEN G. YOUNG,* SUSAN T. NORTHEY, BRIAN J. MCCARTHY

Familial hypobetalipoproteinemia is a syndrome in which the plasma levels of apolipoprotein B (apo-B) and cholesterol are abnormally low. A truncated species of apo-B was identified in the plasma lipoproteins of members of a kindred with familial hypobetalipoproteinemia. DNA sequencing studies on genomic clones and enzymatically amplified genomic DNA samples revealed a four–base pair deletion in the apo-B gene. This short deletion, which results in a frameshift and a premature stop codon, accounts for the truncated apo-B species and explains the low apo-B and low cholesterol levels in this family.

FAMILIAL HYPOBETALIPOPROTEINEmia is a condition in which the concentrations of apolipoprotein B (apo-B) and low density lipoprotein (LDL)–cholesterol in the plasma are abnormally low (*1*). In the homozygous form, apo-B and LDL-cholesterol are either absent from the plasma or present in extremely low concentrations. Homozygotes may have multiple medical problems, including fat malabsorption and neurological disorders. Heterozygotes have apo-B and LDL-cholesterol levels approximately half those of normal; these individuals are usually asymptomatic and may actually be protected from premature atherosclerotic disease (*1*).

In 1979, Steinberg and co-workers described a unique kindred with familial hypobetalipoproteinemia (*2*). Recently, our laboratory identified two different apo-B alleles associated with hypobetalipoproteinemia in that kindred (*3*). One apo-B allele yields a truncated apo-B species, apo-B37, whereas the other allele is associated with low plasma levels of the normal apo-B species, apo-B100. Three individuals had both abnormal alleles and were therefore compound heterozygotes for hypobetalipoproteinemia. There were six heterozygotes with apo-B37, and ten with low levels of apo-B100. The mean LDL-cholesterol level in the latter two groups was 31 mg/dl, less than half of the normal value, whereas it was 6 mg/dl in the compound heterozygotes (*3*).

Because apo-B37 is associated with low

Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA 94140-0608.

*To whom correspondence should be addressed.

plasma cholesterol levels, we were interested in determining its structure. On polyacrylamide gels, apo-B37 appears to be about 37% as large as apo-B100, which contains 4536 amino acids (*4*). Because our earlier protein sequencing and immunochemical studies had indicated that apo-B37 contains only the amino-terminal portion of apo-B100 (*5*), we estimated that the carboxyl-terminal amino acid of apo-B37 must be in the vicinity of apo-B100 amino acid residues 1650–1750. Recent studies showed that an antiserum to a synthetic peptide containing apo-B100 amino acids 1712–1728 bound to apo-B37, enabling us to refine our estimate of the carboxyl terminus of apo-B37 to the vicinity of apo-B100 amino acids 1725–1750. This region of apo-B is encoded by the first third of exon 26 of the apo-B gene (*6*). Southern blots of genomic DNA from apo-B37 heterozygotes revealed no detectable abnormality in this region of the apo-B gene.

We next cloned and sequenced the relevant portion of the apo-B gene of a subject heterozygous for the apo-B37 mutation (that is, he had one normal apo-B allele and one apo-B37 allele) (*7*). Nine clones containing the 3.7-kb Eco RI–Eco RI insert encompassing the first third of exon 26 were selected from a lambda genomic library; each clone was subcloned into M13 for sequencing (*8*). In three clones, there was a 4-bp deletion (apo-B cDNA nucleotides 5391–5394) beginning at the codon for apo-B100 amino acid 1728 (Fig. 1B). The other six clones contained the normal apo-B sequence (Fig. 1A). In both the normal and mutant clones, sequencing of over 500 nucleotides upstream and downstream from the mutation showed no other discrepancies from the consensus apo-B sequence (*4, 6*).

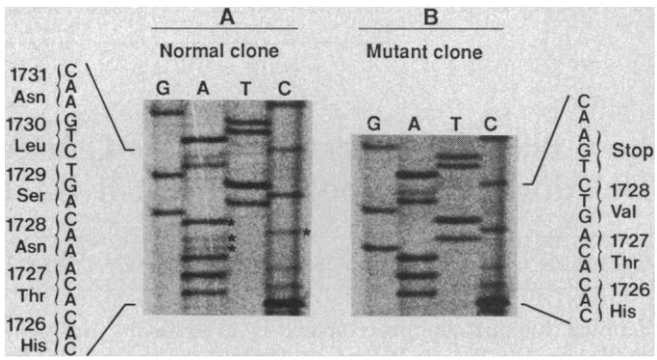

Fig. 1. Autoradiograms of sequencing gels of (**A**) a normal apo-B clone and (**B**) a mutant apo-B clone. The four nucleotides marked by asterisks in the normal clone are those deleted in the mutant clone.

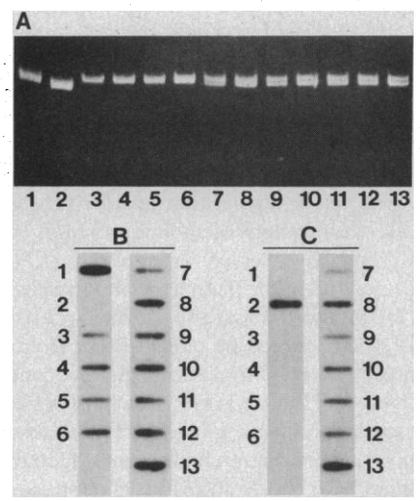

Fig. 2. Amplification of the region of the apo-B gene flanking the apo-B37 mutation. Amplification of genomic DNA was performed with the *Thermus aquaticus* DNA polymerase, according to instructions of the Geneamp kit (Perkin-Elmer Cetus, part No. N801-0043), with oligonucleotide primers B37-8 (5′-AGTCtAGAAGGACTTAAGCTCTC-3′, apo-B cDNA nucleotides 5322–5344) and B37-9 (5′-TAAAgCTTGTCAGAGCTGTAAAT-3′, complementary to apo-B cDNA nucleotides 5467–5445). A single base substitution (lower case letters) was introduced into the 5′ end of B37-8 and B37-9 to create Xba I and Hind III restriction endonuclease sites, respectively. (**A**) One-fifth of the amplification reaction was loaded onto a 7% polyacrylamide gel, which was stained with ethidium bromide. (Lane 1) The 146-bp fragment amplified from a plasmid containing the normal 3.7-kb Eco RI–Eco RI apo-B insert; (lane 2) the 142-bp fragment amplified from a plasmid containing the mutant 3.7-kb Eco RI–Eco RI apo-B insert; (lane 3) the amplified genomic DNA of subject 10, a normolipidemic family member [see family pedigree (*3*)]; (lanes 4 to 6) the amplified DNA from other control subjects; (lanes 7 to 13) amplified DNA from seven apo-B37 heterozygotes [subjects 1, 18, 13, 34, 28, 3, and 32, respectively, (*3*)]. (**B** and **C**) One-twentieth of each of the 13 amplification reactions was loaded onto nitrocellulose membranes using a slot blot apparatus; the nitrocellulose membranes were then probed with ^{32}P-labeled oligonucleotides specific for the normal or mutant allele (*11*). (B) The autoradiogram of the slot blot probed with B37-2 (5′-CCACACAAACAGTCTGAAC-3′), which is complementary to the normal apo-B sequence; (C) the autoradiogram of the slot blot probed with B37-10 (5′-GACCACACAGTCTGA-3′), which is complementary to the mutant sequence. The slot blots were incubated with the labeled probes for 16 hours at 37°C, washed at 48°C for 1 hour in 2× standard saline citrate (*11*), and then exposed to x-ray film for 45 minutes.

The 4-bp deletion results in a frameshift and one novel amino acid, valine, followed by a premature stop codon. Thus, apo-B37 has 1728 amino acids; the calculated molecular weight of apo-B37 is ~193,000, 37.6% of that of apo-B100 (~513,000).

We used the polymerase chain reaction technique (*9*) to demonstrate the same short deletion in other family members. The region of the apo-B gene flanking the observed 4-bp deletion was enzymatically amplified from genomic DNA. When genomic DNA from control subjects was amplified and then subjected to polyacrylamide gel electrophoresis, a single band was observed (Fig. 2A, lanes 3 to 6). The DNA in these bands was subcloned into M13 and was shown to have the normal apo-B sequence. When genomic DNA from seven different apo-B37 heterozygotes were amplified, a "doublet" band was invariably observed (Fig. 2A, lanes 7 to 13). The DNA in both the upper and lower band of the doublet was sequenced; the DNA in the upper band had the normal apo-B sequence, whereas the DNA in the lower band had the same 4-bp deletion identified in the lambda clones. The 4-bp deletion in the amplified DNA was easily detectable on slot blots with allele-specific oligonucleotide probes (Fig. 2, B and C).

The 4-bp deletion in the apo-B gene accounts for the formation of apo-B37. The low LDL-cholesterol levels in subjects with apo-B37 are explained in part by the apparent inability of apo-B37 to form a cholesteryl ester–enriched particle in the LDL density range (*3, 5, 10*). However, apo-B37 itself is present in very low levels in the plasma of affected subjects. Since apo-B37 cannot bind to the LDL receptor (*10*), apo-B37–containing lipoproteins might be expected to be cleared slowly from the plasma and therefore to be found in a high concentration. The low concentration of apo-B37 in plasma may therefore be attributable to reduced synthesis and secretion of this mutant protein from hepatocytes and enterocytes. As noted above, we have identified a second apo-B allele causing hypobetalipoproteinemia (*3*). Further study of that allele may reveal a new mechanism that can account for the low plasma levels of apo-B and cholesterol.

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28 March 1988; accepted 6 June 1988

Conversion of Normal Behavior to Shiverer by Myelin Basic Protein Antisense cDNA in Transgenic Mice

MOTOYA KATSUKI, MASAHIRO SATO,* MINORU KIMURA, MINESUKE YOKOYAMA, KIMIO KOBAYASHI, TATSUJI NOMURA

Myelin basic proteins (MBPs) are coded by the single gene necessary for myelin formation in the central nervous system of the mouse. An antisense MBP mini-gene was constructed and used to determine the function of antisense DNA in transgenic mice. Several transgenic offspring of a founder transgenic mouse, AS100, were converted from the normal to mutant shiverer phenotype. Antisense MBP messenger RNA was expressed in these mice, and the endogenous MBP messenger RNA, the MBP, and the myelination in the central nervous system were reduced.

ANTISENSE RNA (MINUS-STRAND RNA complementary to a particular RNA) has been shown to repress the expression of specific genes in cells of some species including mammals (*1*–*7*). The expression of these antisense RNAs and the repression of the targeted gene functions in transgenic animals provide a means for studying the biological functions of cloned genes.

We chose the myelin basic protein (MBP) gene for repression by the antisense DNA for two reasons: (i) the MBP gene promoter affects the expression of MBP cDNA, tissue specifically, in transgenic shiverer mouse brain and can rescue the shiverer phenotype (*8*), and (ii) an MBP-deficient and hypomyelinating mouse that has an autosomal recessive mutation in the MBP gene, the shiverer (*9*–*13*), is available for comparisons with transgenic mice having the antisense MBP DNA.

To examine the myelination in the transgenic mice having antisense MBP DNA, we constructed a plasmid pMP302AS. This plasmid contained the mouse MBP promoter region (approximately 1.3 kb) followed by a portion of the rabbit β-globin gene carrying the mouse MBP cDNA for the smallest MBP (14 kD) in the antisense orientation and by the polyadenylation sites of rabbit β-globin and simian virus 40 (SV40) early genes (Fig. 1A). The DNA fragment, antisense MBP mini-gene, that was injected into mouse zygotes was prepared from plasmid pMP302AS DNA that had been digested with Hind III and Sal I. Fertilized eggs heterozygous for the shiverer mutation (*shi*/+) were used to produce the transgenic mice. Inhibition of the endogenous sense MBP mRNA expression by the antisense RNA is expected to be more pronounced in heterozygous (*shi*/+) than in wild-type mice (+/+), as the amount of MBP mRNAs in the heterozygous mouse is half that in the wild type (*14*). The method of producing transgenic mice was basically that described by Gordon *et al.* (*15*). Five transgenic mice with the antisense MBP mini-gene were obtained. All five founder mice, each of which had been independently produced, appeared to be normal.

The transgenic male mouse, AS100 (*shi*/+), was mated with a wild-type female mouse (+/+, B6c F1 hybrid) and 50 offspring were born (AS100-1 to AS100-50). The transgene of the AS100 mouse was transmitted to 21 of them. There were two types of transgene, A and B. The A type had extra bands as well as the B-type bands as shown by DNA blot analyses (Fig. 1B). These two types were segregated to A and B strains and stably transmitted to their offspring. There also were two different genetic backgrounds for these offspring, *shi*/+ and +/+, shown by the presence of the truncated restriction fragment found only in the heterozygous shiverer mouse (*shi*/+) (*13*) because of the large deletion of the MBP gene in shiverer mutation (*10*, *11*).

Surprisingly, 10 of the 21 transgenic mice began to show shivering at about 2 weeks after birth. There was some variation in the severity of the tremors, which became progressively pronounced. Further analysis of the AS100 transgenic offspring showed that

Table 1. Number of transgenic mice with the tremor phenotype in relation to genetic background. A and B represent the two types of transgene in the offspring of AS100 mouse.

Genotype	Phenotype	
	Tremor	Normal
shi/+ (A, B)	7 (3, 4)	5 (3, 2)
+/+ (A, B)	3 (1, 2)	6 (0, 6)
Total (A, B)	10 (4, 6)	11 (3, 8)

Table 2. Correlation between MBP expression and the tremor phenotype in transgenic mice.

Mouse	Genetic background	Transgene*	Antisense MBP mRNA†	Endogenous MBP mRNA (%)‡	MBP in cerebellum§	Tremor phenotype‖
AS100-10	+/+	−	−	100	+	−
AS100-9	*shi*/+	−	−	50	+	−
AS100-11	+/+	B type	+	50	+	−
AS100-12	*shi*/+	A type	++	30	± (m)	±
AS100-14	*shi*/+	B type	++	20	∓ (m)	+

*A and B type represent the types of transgene transmitted; − indicates a nontransgenic mouse. †Antisense MBP mRNA in the brain was detected by RNA blot analysis with sense MBP RNA as the probe. ‡Relative amount of MBP mRNA in the brain, the wild type (+/+) being taken as 100%. §+ indicates that the MBP was detected uniformly in the cerebellar tissue by anti-MBP; ± indicates relatively sparse distribution of MBP; ∓ indicates intermediate distribution of MBP between − and ±; and m means mosaic expression of MBP. ‖− indicates normal behavior; + indicates tremor phenotype; and ± indicates the faint tremor phenotype.

M. Katsuki, Department of DNA Biology, School of Medicine and Institute of Medical Sciences, Tokai University, Bohseidai, Isehara 259-11, Japan, and Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki 213, Japan.
M. Sato and M. Kimura, Department of DNA Biology, School of Medicine and Institute of Medical Sciences, Tokai University, Bohseidai, Isehara 259-11, Japan.
M. Yokoyama, K. Kobayashi, T. Nomura, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki 213, Japan.

*On leave from the R&D Laboratories of Hoechst Japan Ltd., Minamidai, Kawagoe 350, Japan.