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Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein(a)

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ABSTRACT **Lipoprotein(a) [Lp(a)] is a lipoprotein formed by the disulfide linkage of apolipoprotein (apo) B100 of a low density lipoprotein particle to apolipoprotein(a). Prior studies have suggested that one of the C-terminal Cys residues of apo-B100 is involved in the disulfide linkage of apo-B100 to apo(a). To identify the apo-B100 Cys residue involved in the formation of Lp(a), we constructed a yeast artificial chromosome (YAC) spanning the human apo-B gene and used gene-targeting techniques to change Cys-4326 to Gly. The mutated YAC DNA was used to generate transgenic mice expressing the mutant human apo-B100 (Cys4326Gly). Unlike the wild-type human apo-B100, the mutant human apo-B100 completely lacked the ability to bind to apo(a) and form Lp(a). This study demonstrates that apo-B100 Cys-4326 is required for the assembly of Lp(a) and shows that gene targeting in YACs, followed by the generation of transgenic mice, is a useful approach for analyzing the structure of large proteins coded for by large genes.**

Lipoprotein(a) [Lp(a)] is a lipoprotein (1) formed by the covalent linkage of apolipoprotein (apo) B100 of a low density lipoprotein (LDL) particle to apo(a) (2). In many human studies, high plasma levels of Lp(a) have been found to be associated with an increased risk of atherosclerotic coronary heart disease (3, 4), although several recent studies have not been able to detect this association (5, 6).

There is strong evidence that Lp(a) formation depends on a disulfide bond between Cys residues in apo-B100 and apo(a). Disulfide reducing agents dissociate Lp(a) into apo(a) and apo-B100 (7), and site-directed mutagenesis studies on apo(a) have shown that apo(a) Cys-4057 is required for the formation of Lp(a) (8, 9). To date, however, the identity of the Cys within apo-B100 that is involved in the formation of Lp(a) has not been determined. Molecular modeling studies (10), immunochemical studies with peptide-specific antisera (11), and apo-B100-labeling studies with fluorescent sulfhydryl compounds (12) have suggested the possibility that apo-B100 Cys-3734 might be involved in Lp(a) formation. However, these indirect approaches have not definitively identified the apo-B100 Cys involved in the disulfide linkage. Recently, studies of truncated apo-B proteins have suggested that the C-terminal portion of apo-B100 is essential for the formation of Lp(a). Our laboratory demonstrated that human apo-B90 (4084 aa long) was incapable of binding to apo(a) to form Lp(a) (13); in addition, Gabel *et al.* (14) found that apo-B94 (4270 aa) lacked the capacity to form Lp(a). These studies strongly suggested that sequences within the C-terminal 10% of the apo-B molecule were required for the formation of Lp(a).

In this study, we tested the hypothesis that the C-terminal Cys of apo-B100 Cys-4326 is required for Lp(a) formation. Cys-4326 is one of two Cys residues in the C-terminal 10% of apo-B100 (the other being Cys-4190), and it is the only Cys not present in the apo-B94 construct analyzed by Gabel *et al.* (14). To test the hypothesis that Cys-4326 is the critical Cys residue, we sought to express a mutant human apo-B100 lacking Cys-4326 in transgenic mice. We cloned a 79.5-kb fragment of human genomic DNA spanning the human apo-B gene (15) into a yeast artificial chromosome (YAC) vector and then used gene targeting to mutate the codon for apo-B100 Cys-4326. The YAC DNA was then used to generate transgenic mice expressing the mutant human apo-B100. Unlike the wild-type human apo-B100, the mutant human apo-B100 completely lacked the capacity to bind to apo(a) to form Lp(a).

MATERIALS AND METHODS

Cloning of the apo-B YAC. An 87-kb *Mlu* I fragment from p158 (15), a P1 bacteriophage clone spanning the human apo-B gene, was ligated into the YAC vector pYACRC (American Type Culture Collection), as described by McCormick *et al.* (16). The 99-kb ligation product was used to transform yeast spheroplasts made from the yeast strain AB1380 (17). All of the *TRP1 URA3* transformants contained a 99-kb YAC. The *URA3* gene in the right YAC arm was replaced with a *LYS2* gene by retrofitting with a 9-kb insert from pRV1, which also contains a neomycin-resistance gene (18); this modification extended the length of the YAC to 108 kb.

Mutagenesis of the apo-B YAC. To mutate the apo-B YAC, we used the "pop-in, pop-out" gene-targeting strategy (19, 20) illustrated in Fig. 1. The gene-targeting vector was constructed by cloning a 2.8-kb *Xba* I fragment spanning from intron 28 to exon 29 of the human apo-B gene into the yeast integrating vector pRS406, which contains the *URA3* gene (Stratagene). To change the Cys-4326 codon to a Gly codon, site-directed mutagenesis was performed by using the oligonucleotide GAAAGAAAACCTAgGCCTTAATCTTCATAAG. The single-nucleotide substitution (lowercase type) also created a new *Stu* I site. After DNA sequencing, the gene-targeting vector was linearized at the *Eco*RI site in exon 29 and introduced into AB1380 spheroplasts containing the 108-kb apo-B YAC. Transformants were initially selected on plates lacking uracil and later grown on plates lacking uracil, Trp, and Lys. To identify targeted clones (the pop-in step), yeast colonies were analyzed by pulsed-field gel electrophoresis and Southern blot analysis as described below. For the pop-out

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Abbreviations: YAC, yeast artificial chromosome; Lp(a), lipoprotein(a); apo, apolipoprotein; LDL, low density lipoprotein.

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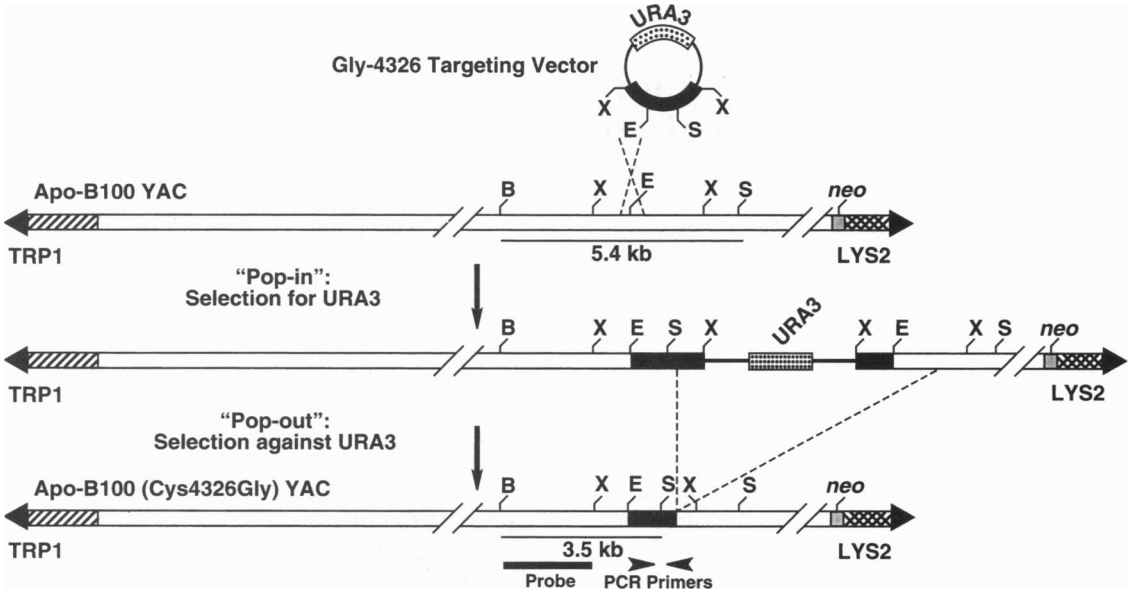

FIG. 1. (*A*) Strategy for introducing the Cys4326Gly mutation into the human apo-B100 YAC. The Gly-4326 gene-targeting vector was linearized at the *Eco*RI site in exon 29 of the apo-B gene and used to transform yeast spheroplasts. In the pop-in step, the recombination of the targeting vector with the YAC apo-B gene yields a larger YAC containing the entire gene-targeting vector. In the pop-out step, intrachromosomal recombination between the homologous apo-B gene sequences deletes the vector sequences, the *URA3* gene, and the duplicated apo-B gene sequences but leaves the targeted mutation in the apo-B gene. B, *Bam*HI; X, *Xba* I; E, *Eco*RI; S, *Stu* I; neo, neomycin-resistance gene. The location of the probe and PCR primers used for characterizing YAC gene targeting events are shown.

step, yeast harboring a targeted YAC were grown in medium lacking Lys and Trp overnight and then plated onto 5-fluoroorotic acid plates (21). The colonies were subsequently plated onto plates lacking Trp and Lys and examined by pulsed-field gel electrophoresis, Southern blot, and PCR analyses, as described below.

Analysis of the Gene-Targeting Events in the YACs. High molecular weight yeast DNA (22) was electrophoresed on a 1% pulsed-field agarose gel in 0.5× TBE (1× = 90 mM Tris/90 mM boric acid/2 mM EDTA, pH 8.3) at 6 V/cm for 15.16 hr at 14°C, with initial and final switching times of 0.22 and 21.79 sec, respectively. To identify gene-targeting events, we performed Southern blot analysis on yeast genomic DNA that had been cleaved with *Bam*HI and *Stu* I. The digested DNA was resolved on a 1% agarose gel, blotted onto a nylon membrane, and then hybridized with a 1.7-kb *Bam*HI–*Xba* I apo-B gene probe located 5′ to sequences contained in the gene-targeting vector (see Fig. 1). To confirm mutagenesis of the YAC, a 354-bp segment of exon 29 of the apo-B gene (apo-B cDNA nt 13,067–13,421) was enzymatically amplified from yeast DNA (using primers 5′-CATTAAACAGCTGAAAGAGATGA-3′ and 5′-GTTAGAGGCACTGACAATATATTC-3′), cleaved with *Stu* I, and analyzed on an ethidium bromide-stained 10% polyacrylamide gel.

Generation of Transgenic Mice. To generate transgenic mice expressing the mutant apo-B100 (Cys4326Gly), the 108-kb YAC was purified from pulsed-field agarose gels as described (22), adjusted to a concentration of 3 ng/μl, and microinjected into C57BL/6 × SJL mouse zygotes. Transgenic founders were identified by analysis of tail DNA for the mutant sequence and of plasma for the presence of human apo-B100 by using Western blots and a specific RIA (15).

Characterization of the Mutant Human apo-B100 in Transgenic Mouse Plasma. The ability of multiple human apo-B-specific monoclonal antibodies to bind to the mutant apo-B100 in transgenic mouse plasma was examined by Western blot analysis of 4% polyacrylamide/SDS gels with the following apo-B-specific monoclonal antibodies: 1D1 (which binds between apo-B100 aa 474 and 539), 2D8 (which binds between aa 1438 and 1480), MB44 (which binds near aa 2500), 4G3 (which binds between aa 2980 and 3084), MB47 (which binds near aa 3500), MB43 (which binds between aa 4027 and 4081), Bsol16 (which binds between aa 4157 and 4189), and Bsol7 (which binds between aa 4521 and 4536) (23). As controls for these experiments, we used plasma samples from transgenic animals that expressed high levels of wild-type human apo-B100 (transgenic line 1102 from ref. 15) or apo-B90 (13). Plasma from transgenic founder animals expressing the mutant human apo-B100 (Cys4326Gly) and from transgenic mice expressing the wild-type human apo-B100 (15) was size-fractionated by gel filtration chromatography on a Superose 6 10/30 column (15).

Assessing the Ability of the Mutant apo-B100 to Bind to apo(a) to Form Lp(a). The ability of the mutant apo-B100 to bind to apo(a) was assessed in the Western blot assay described by Chiesa *et al.* (24) and modified by McCormick *et al.* (13). The plasma from an apo(a) transgenic mouse (24) was used as a source of free apo(a). Plasma from the apo(a) transgenic mouse was incubated with the following plasma samples: plasma from a wild-type human apo-B100 transgenic mouse, plasma from a human apo-B90 transgenic mouse, and plasma from a transgenic founder animal expressing the mutant human apo-B100 (Cys4326Gly). All incubation mixtures contained identical amounts of human apo-B, as judged by specific RIAs and Western blot analysis using the human apo-B-specific monoclonal antibody 1D1 (23). Aliquots of each of the incubation mixtures were resolved on 4% polyacrylamide/SDS gels under nonreducing and reducing [3% (vol/vol) 2-mercaptoethanol] conditions and then transferred to nitrocellulose for Western blot analysis with the human apo(a)-specific monoclonal antibody IgG-1A2. The relative amounts of Lp(a) formed in each incubation mixture were also assessed by using a monoclonal antibody-based solid-phase RIA (13). Finally, we bred mice expressing the mutant apo-B with apo(a) transgenic mice (24) and examined the plasma of the double transgenic offspring for the presence of Lp(a) by Western blot analysis.

RESULTS

We sought to express a mutant human apo-B100 lacking Cys-4326 and determine whether the mutant apo-B100 re-

tained the ability to bind to apo(a) to form Lp(a). To introduce a subtle mutation into the apo-B gene, we ligated an 87-kb fragment from a P1 clone into a YAC vector and then used a gene-targeting strategy to change the codon for apo-B100 Cys-4326 to Gly (Fig. 1). A sequence insertion gene-targeting vector was constructed, linearized within the segment of homology, and transfected into yeast spheroplasts containing the 108-kb apo-B YAC. After the pop-in step, pulsed-field agarose gel electrophoresis revealed that the YAC had increased in size by 7 kb (Fig. 2*A*, lane 3). To document that the targeting vector had integrated into the homologous segment of the human apo-B gene, we performed Southern blot analysis of yeast genomic DNA. Because the targeted amino acid substitution was associated with a new *Stu* I site, correct integration of the vector could be identified by the presence of a 3.5-kb *Bam*HI–*Stu* I fragment (Fig. 2*B*, lane 3). The gene-targeting process was completed in the pop-out step by growing the yeast in 5-fluoroorotic acid, which selects for the loss of the *URA3* gene by intrachromosomal recombination between the homologous apo-B gene segments (Fig. 1). After the pop-out step, pulsed-field gels showed that the YAC had returned to its original size (Fig. 2*A*, lane 4), and Southern blot analysis confirmed the retention of the targeted *Stu* I site (Fig. 2*B*, lane 4). The presence of the targeted mutation in the YAC DNA was also confirmed by enzymatically amplifying a 354-bp segment of DNA flanking the mutation and showing that it was completely cleaved with *Stu* I (Fig. 2*C*, lane 4). The percentages of yeast colonies that had undergone the correct homologous recombination events in the pop-in and pop-out steps were ≈40% and ≈25%, respectively.

The YAC DNA was purified from pulsed-field agarose gels and microinjected into mouse zygotes to generate transgenic mice. From 136 mice, we identified 17 transgenic founders expressing the mutant human apo-B (Cys4326Gly) in their plasma. The genomic DNA of the founders contained the new *Stu* I site, as assessed by PCR analysis (data not shown). Fig. 3*A* shows the ability of three human apo-B-specific monoclonal antibodies (1D1, MB44, and Bsol7) to bind to the mutant human apo-B100. To analyze the distribution of the mutant apo-B in the plasma of the transgenic mice, plasma from one of the transgenic founder animals was fractionated on a Superose 6 10/30 column. The distribution of apo-B in mice expressing the mutant and wild-type human apo-B100 was identical, with most residing in the LDL fraction (Fig. 3*B*). Mice expressing the mutant human apo-B100 had a prominent LDL cholesterol peak, similar to that observed in mice expressing wild-type human apo-B100 (Fig. 3*C*).

The ability of the mutant apo-B100 to form Lp(a) was assessed by using a Western blot assay (24). The plasma from an apo(a) transgenic mouse (24) was incubated with the plasma samples from transgenic mice expressing wild-type human apo-B100, human apo-B90, and the mutant human apo-B100 (Cys4326Gly). The incubation mixtures were then size-fractionated on SDS/polyacrylamide gels under nonreducing conditions, and Western blot analysis was performed with an apo(a)-specific monoclonal antibody. The formation of Lp(a) could be identified readily because Lp(a) is much larger than free apo(a). As expected, the wild-type human apo-B100 bound all of the apo(a) in the incubation mixture and formed Lp(a) (Fig. 4*A*, lane 2). In contrast, no Lp(a) was formed during the incubation of apo(a) and the mutant human apo-B100 (Fig. 4*A*, lane 4). Similarly, no Lp(a) was formed during the incubation with human apo-B90 (Fig. 4*A*, lane 3). As expected, no Lp(a) was detected in any of the incubation mixtures when they were incubated with a disulfide reducing agent prior to gel electrophoresis (Fig. 4*B*). The formation of Lp(a) was also assessed by using a solid-phase monoclonal antibody-based sandwich RIA (13). Consistent with the Western blot results, the solid-phase RIA revealed that the wild-type human apo-B100, but not the mutant apo-B100, formed Lp(a) (Fig. 4*C*, solid bars). The relative amount of apo-B100 in each incubation mixture was equivalent, as shown by an apo-B RIA (Fig. 4*C*, striped bars). The inability of the mutant apo-B100 to form Lp(a) was documented in experiments using the plasma from three founder animals expressing the mutant human apo-B100.

One of the founder animals was mated with an apo(a) transgenic mouse (24); one of the offspring expressed both apo(a) and the mutant human apo-B100. By Western blot analysis, Lp(a) was undetectable in the plasma of that double-transgenic mouse (data not shown). In contrast, mice expressing both apo(a) and wild-type human apo-B100 have abundant Lp(a) in their plasma (15).

DISCUSSION

This laboratory has used (15) a 79.5-kb insert from a P1 bacteriophage clone to generate transgenic mice expressing high levels of human apo-B100 and has shown that the human apo-B100 in the plasma of the transgenic animals bound to

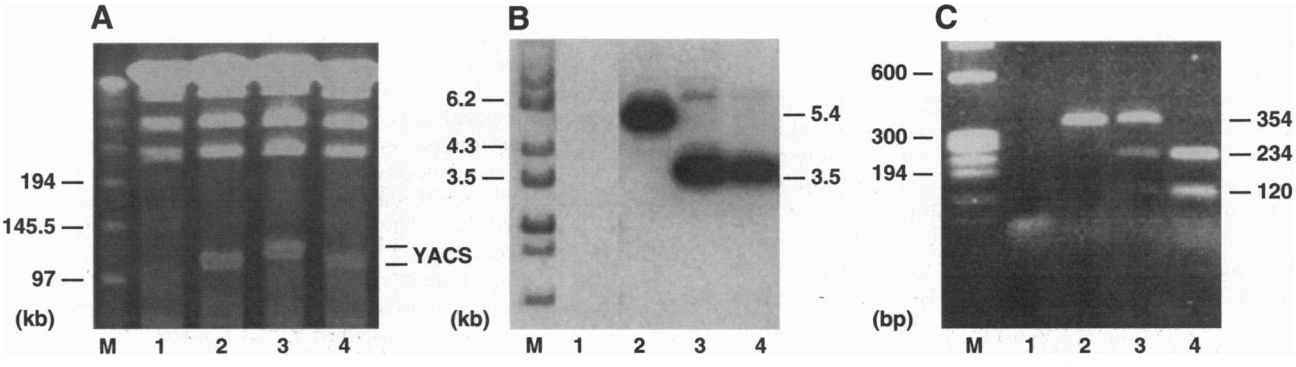

FIG. 2. (*A*) Pulsed-field gel analysis of the YAC gene-targeting events. High molecular weight yeast DNA was separated on a 1% agarose pulsed-field gel and stained with ethidium bromide. Lanes: M, low-range pulsed-field gene DNA markers from New England Biolabs; 1, yeast strain AB1380; 2, AB1380 containing the wild-type apo-B YAC; 3, AB1380 containing the pop-in Cys4326Gly apo-B YAC; 4, AB1380 containing the pop-out Cys4326Gly apo-B YAC. Integration of the vector in the pop-in step adds 7 kb to the size of the YAC, whereas intrachromosomal recombination in the pop-out step returns the YAC to its original size. (*B*) Southern blot analysis of the YAC gene-targeting events. Yeast genomic DNA was digested with *Bam*HI and *Stu* I and examined by Southern blot analysis with an apo-B gene probe located 5′ to the sequences contained in the targeting vector (see Fig. 1). Lanes: M, ^{35}S-labeled DNA markers from Amersham; 1–4, same as in *A*. (*C*) PCR analysis of the YAC gene-targeting events. A 354-bp fragment of the apo-B gene spanning the mutation was enzymatically amplified from yeast DNA, digested with *Stu* I, and analyzed on an ethidium bromide-stained gel. Lanes: M, ϕX174 DNA markers (GIBCO/BRL); 1–4, same as in *A*.

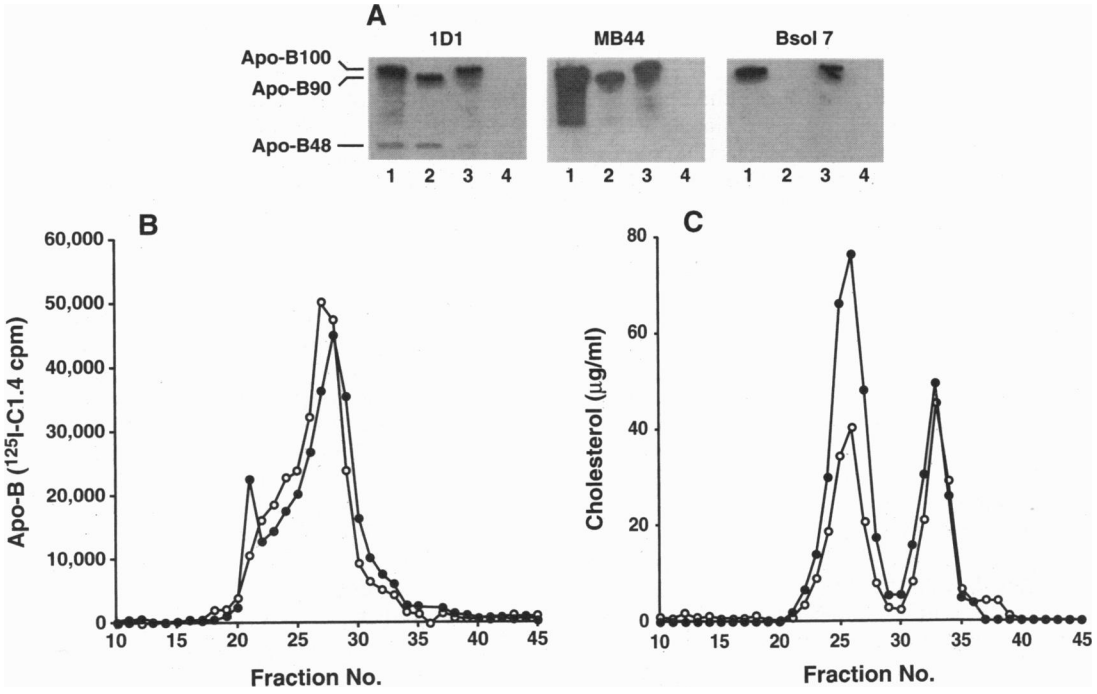

FIG. 3. (*A*) Western blot analysis of transgenic mouse plasma using human apo-B-specific monoclonal antibodies. Lanes: 1, plasma from a wild-type human apo-B100 transgenic mouse; 2, plasma from a human apo-B90 transgenic mouse; 3, plasma from a transgenic mouse expressing the mutant human apo-B100 (Cys4326Gly); 4, plasma from a nontransgenic mouse. In separate experiments, the mutant apo-B was also bound by monoclonal antibodies MB47, MB43, Bsol16, and 4G3 (data not shown). (*B*) Distribution of human apo-B100 within the plasma of female transgenic mice expressing either wild-type human apo-B100 (solid circles) or mutant human apo-B100 (Cys4326Gly) (open circles). Plasma was fractionated on a Superose 6 10/30 column. The amount of human apo-B in each column fraction was assessed in an RIA using C1.4, an antibody specific for human apo-B (13). The ^{125}I-labeled C1.4 (^{125}I-C1.4) cpm in each fraction reflects the relative amount of human apo-B100 in each fraction. (*C*) Distribution of cholesterol within the plasma of female transgenic mice expressing either wild-type human apo-B100 or mutant human apo-B100 (Cys4326Gly). The cholesterol concentration in each Superose 6 column fraction was determined by using a colorimetric assay (15). Fractions: 17–23, very low density lipoprotein- and intermediate density lipoprotein-sized lipoproteins; 24–29, LDL-sized lipoproteins; 30–35, high density lipoprotein-sized lipoproteins. Both animals had triglyceride-enriched LDL (data not shown).

apo(a) to form Lp(a). We then expressed (13) a truncated human apo-B, apo-B90 (4084 aa), in transgenic mice and showed that it lacked the capacity to bind to apo(a). In the present study, we tested the hypothesis that the last Cys in the sequence of apo-B100 Cys-4326 was essential for Lp(a) formation. We constructed a YAC spanning the human apo-B gene, used gene-targeting techniques to change the Cys-4326 codon to a Gly codon, and then used the YAC DNA to generate transgenic mice expressing the mutant human apo-B100. Unlike wild-type human apo-B100, the mutant human apo-B100 completely lacked the capacity to bind to apo(a) to form Lp(a). These data indicate that apo-B100 Cys-4326 is required for the formation of Lp(a), ending long-standing uncertainty regarding the identity of the apo-B100 Cys involved in the disulfide linkage with apo(a). Although it seems overwhelmingly likely that Cys-4326 represents the site of attachment for apo(a), it is worthwhile acknowledging the possibility that the targeted amino acid substitution may have prevented Lp(a) formation by causing a conformational change in the apo-B100 molecule. It is noteworthy that Cys-4326 is not conserved in the sequence of mouse (S.G.Y. and V. Pierotti, unpublished data), rat (25), or pig (26) apo-B100, all of which lack the capacity to bind to apo(a) to form Lp(a) (H. Hobbs, personal communication and ref. 24). It is unknown whether the introduction of Cys-4326 into the apo-B100 of these animals would be sufficient to permit Lp(a) formation or whether the apo-B100 of these animals also lacks other structural features required for the association of apo-B100 with apo(a). The structural features of apo-B100, aside from Cys-4326, that are required for Lp(a) formation are unknown but could be explored experimentally by using the mutagenesis techniques described herein.

The gene targeting in YACs represents a significant tool for analyzing structural features of large proteins encoded by large genes. In the case of the apo-B gene, targeted mutagenesis in YACs, followed by expression studies in transgenic mice, certainly represent a major advance for approaching protein structure–function relationships. In the past, the expression of a mutant human apo-B100 was a formidable task. Minigene expression vectors for apo-B100 have been described (27), but they are large (>20 kb) and have very few unique cloning sites. Consequently, generating a mutant expression plasmid by mutating a small DNA fragment and then reconstructing the full-length minigene expression plasmid is difficult and time-consuming. Moreover, cultured hepatoma cells transfected with the minigene vectors do not secrete high levels of apo-B100 (28), and minigene vectors fitted with heterologous liver-specific promoters yield extremely poor expression in transgenic mice (29, 30). In contrast, gene targeting in YACs eliminates complicated cloning strategies, and the high efficiency of homologous recombination in yeast means that the identification of correctly targeted YACs is a trivial task. Finally, in our studies, microinjection of purified YAC DNA yielded transgenic founders with an efficiency that compares favorably with those obtained with plasmid or P1 DNA. We anticipate that gene targeting in YACs will be useful for studying structure–function relationships in other large proteins encoded by large genes. Furthermore, as shown by Peterson *et al.* (20), gene targeting in YACs will be useful for analyzing the regulatory elements controlling gene expression.

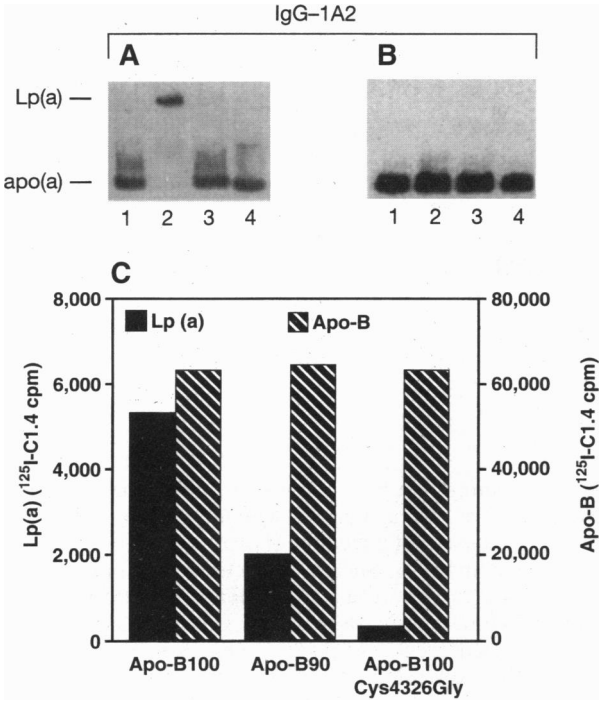

FIG. 4. (*A*) Western blot analysis of Lp(a) formation. The apo(a)–apo-B incubations were resolved on an SDS/polyacrylamide gel under nondenaturing conditions, and Western blot analysis was performed by using an apo(a)-specific monoclonal antibody. Lanes: 1, plasma from the apo(a) transgenic mouse; 2, the incubation mixture containing wild-type human apo-B100; 3, the incubation mixture containing human apo-B90; 4, the incubation mixture containing the mutant human apo-B100 (Cys4326Gly). (*B*) Western blot of the same apo(a)–apo-B incubation mixtures treated with 3% 2-mercaptoethanol prior to gel electrophoresis. Lanes 1–4 are as in *A*. (*C*) Solid-phase RIA analysis of Lp(a) formation. The amount of Lp(a) formed during each incubation was assessed in a sandwich RIA using the apo(a)-specific antibody LPA6 as the "capture" antibody and ^{125}I-labeled C1.4 (specific for human apo-B) as the "detection" antibody (13). The relative amount of human apo-B in each incubation mixture was also assessed in a sandwich RIA, using antibody MB47 (specific for human apo-B100) as the capture antibody and ^{125}I-labeled C1.4 as the detection antibody (13). The bars show the specific ^{125}I-labeled C1.4 cpm bound per well, with all determinations made in triplicate.

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1. Utermann, G. (1989) *Science* **246,** 904–910.
2. McLean, J. W., Tomlinson, J. E., Kuang, W.-J., Eaton, D. L., Chen, E. Y., Fless, G. M., Scanu, A. M. & Lawn, R. M. (1987) *Nature (London)* **330,** 132–137.
3. Kostner, G. M., Avogaro, P., Cazzolato, G., Marth, E., Bittolo-Bon, G. & Quinci, G. B. (1981) *Atherosclerosis* **38,** 51–61.
4. Dahlen, G. H., Guyton, J. R., Attar, M., Farmer, J. A., Kautz, J. A. & Gotto, A. M., Jr. (1986) *Circulation* **74,** 758–765.
5. Jauhiainen, M., Koskinen, P., Ehnholm, C., Frick, M. H., Mänttäri, M., Manninen, V. & Huttunen, J. K. (1991) *Atherosclerosis* **89,** 59–67.
6. Ridker, P. M., Hennekens, C. H. & Stampfer, M. J. (1993) *J. Am. Med. Assoc.* **270,** 2195–2199.
7. Utermann, G., Menzel, H. J., Kraft, H. G., Duba, H. C., Kemmler, H. G. & Seitz, C. (1987) *J. Clin. Invest.* **80,** 458–465.
8. Brunner, C., Kraft, H.-G., Utermann, G. & Müller, H.-J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11643–11647.
9. Koschinsky, M. L., Côté, G. P., Gabel, B. & van der Hoek, Y. Y. (1993) *J. Biol. Chem.* **268,** 19819–19825.
10. Guevara, J., Jr., Spurlino, J., Jan, A. Y., Yang, C.-Y., Tulinsky, A., Prasad, B. V. V., Gaubatz, J. W. & Morrisett, J. D. (1993) *Biophys. J.* **64,** 686–700.
11. Guevara, J., Jr., Valentinova, N. V., Sparrow, J. T., Yang, C.-Y., Xie, Y.-H., Vlasik, T. & Morrisett, J. D. (1993) *Circulation* **88,** I-271 (abstr.).
12. Coleman, R. D., Kim, T. W., Gotto, A. M., Jr. & Yang, C.-Y. (1990) *Biochim. Biophys. Acta* **1037,** 129–132.
13. McCormick, S. P. A., Linton, M. F., Hobbs, H. H., Taylor, S., Curtiss, L. K. & Young, S. G. (1994) *J. Biol. Chem.* **269,** 24284–24289.
14. Gabel, B., McLeod, R. S., Yao, Z., Young, S. G. & Koschinsky, M. L. (1994) *FEBS Lett.* **350,** 77–81.
15. Linton, M. F., Farese, R. V., Jr., Chiesa, G., Grass, D. S., Chin, P., Hammer, R. E., Hobbs, H. H. & Young, S. G. (1993) *J. Clin. Invest.* **92,** 3029–3037.
16. McCormick, M. K. (1994) in *YAC Libraries: A User's Guide*, eds. Nelson, D. L. & Brownstein, B. H. (Freeman, New York), pp. 33–56.
17. Burke, D. T. & Olson, M. V. (1991) *Methods Enzymol.* **194,** 251–270.
18. Srivastava, A. K. & Schlessinger, D. (1991) *Gene* **103,** 53–59.
19. Duff, K., McGuigan, A., Huxley, C., Schulz, F. & Hardy, J. (1994) *Gene Ther.* **1,** 70–75.
20. Peterson, K. R., Li, Q.-L., Clegg, C. H., Furukawa, T., Navas, P. A., Norton, E. J., Kimbrough, T. G. & Stamatoyannopoulos, G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5655–5659.
21. Rothstein, R. (1991) *Methods Enzymol.* **194,** 281–301.
22. Peterson, K. R., Clegg, C. H., Huxley, C., Josephson, B. M., Haugen, H. S., Furukawa, T. & Stamatoyannopoulos, G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7593–7597.
23. Pease, R. J., Milne, R. W., Jessup, W. K., Law, A., Provost, P., Fruchart, J.-C., Dean, R. T., Marcel, Y. L. & Scott, J. (1990) *J. Biol. Chem.* **265,** 553–568.
24. Chiesa, G., Hobbs, H. H., Koschinsky, M. L., Lawn, R. M., Maika, S. D. & Hammer, R. E. (1992) *J. Biol. Chem.* **267,** 24369–24374.
25. Reuben, M. A., Svenson, K. L., Doolittle, M. H., Johnson, D. F., Lusis, A. J. & Elovson, J. (1988) *J. Lipid Res.* **29,** 1337–1347.
26. Purtell, C., Maeda, N., Ebert, D. L., Kaiser, M., Lund-Katz, S., Sturley, S. L., Kodoyianni, V., Grunwald, K., Nevin, D. N., Aiello, R. J. & Attie, A. D. (1993) *J. Lipid Res.* **34,** 1323–1335.
27. Blackhart, B. D., Yao, Z. & McCarthy, B. J. (1990) *J. Biol. Chem.* **265,** 8358–8360.
28. Heinemann, T., Metzger, S., Fisher, E. A., Breslow, J. L. & Huang, L.-S. (1994) *J. Lipid Res.* **35,** 2200–2211.
29. Chiesa, G., Johnson, D. F., Yao, Z., Innerarity, T. L., Mahley, R. W., Young, S. G., Hammer, R. H. & Hobbs, H. H. (1993) *J. Biol. Chem.* **268,** 23747–23750.
30. Young, S. G., Farese, R. V., Jr., Pierotti, V. R., Taylor, S., Grass, D. S. & Linton, M. F. (1994) *Curr. Opin. Lipidol.* **5,** 94–101.