The Influence of Chromosomal Location on the Expression of Two Transgenes in Mice

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We have generated mice having a single copy of the human haptoglobin gene (Hp), driven by its natural promoter, and a neomycin resistance gene (Neo), driven by a herpes simplex thymidine kinase promoter with polyoma enhancers, inserted into two defined chromosomal locations, the Hprt locus on the X-chromosome and the apolipoprotein (apo) AI-CIII gene cluster on chromosome 9. The haptoglobin promoter is highly specialized in its tissue of action; the viral promoter has few restrictions. The apoAI-CIII gene is naturally active in only two tissues, whereas the Hprt gene region is ubiquitously active. Expression of both transgenes at substantial levels was achieved (a) when the transgenes were inserted into the genome close to a known tissue-specific enhancer/locus control region in the apoAI-CIII gene cluster, and (b) when known conditions for function of their promoters were met. The specificities of the two chromosomal regions and of the two promoters are preserved, but their interactions are tissue-specific. We conclude that transgenes are affected by locus-related enhancers in the same manner as nearby endogenous genes. Our experiments reinforce the usefulness of using gene targeting to direct single-copy transgenes to appropriate chromosomal locations.

Transgenic mouse technology has been widely and effectively used for studying the physiological consequences of expressing exogenous gene products or analyzing promoter function and protein structure-function relationships in vivo (1). However, analysis with transgenic mice produced by pronuclear microinjection requires multiple founders because a foreign gene incorporated into the mouse genome is often expressed differently, both in amount and in tissue specificity in one founder versus another. Integration of the exogenous DNA into random chromosomal locations and in unpredictable numbers of copies, together with rearrangements in the introduced DNA or in the sites of incorporation associated with the integration event, are among the factors accounting for these variations (2, 3).

We have previously described a general way of introducing single-copy transgenes into the mouse germ line via embryonic stem (ES) cell technology as a means of comparing the expression of different transgenic sequences without the complications of variation in copy number and insertion site (4). In this earlier work, we tested the method by inserting into the hypoxanthine phosphoribosyltransferase (Hprt) locus on the X-chromosome a single copy of a murine Bcl-2 cDNA driven either by a chicken β-actin promoter or by a human β actin promoter and demonstrated that expression of the two transgenes differed in a predictable fashion. The Hprt gene is a housekeeping gene, transcriptionally active in all tissues, and modification of the locus does not affect the overall development and health of the animals. Furthermore, the availability of Hprt ES cells, such as E14TG2a (5) and HM-1 (6) and of vectors capable of mediating highly efficient and directly selectable homologous recombination at the Hprt locus makes this system simple to use. The previous work, however, also showed that despite expression of the Hprt locus in all tissues, expression of the Bcl-2 transgene driven by the human or the chicken β actin promoter was very low or undetectable in the liver and kidney; this has prompted us to search for a locus in which liver-specific expression of a targeted transgene can be readily studied.

A gene cluster on mouse chromosome 9, which contains genes, coding for apolipoprotein (apo) A-I, apoC-III, and apoA-IV is a potential target for such studies. The region is relatively easily targeted (7), and alteration of a single copy of any of the three genes in the locus has physiologically insignificant consequences that do not affect the development or overall health of the resulting mice (8). All three apolipoprotein genes are expressed highly in the liver and small intestine, and the promoter/enhancer elements controlling the expression of these genes are well characterized in humans.

In the present study, we tested the expectation that the choice of the site of transgene insertion will cause a predictable influence on the tissue-specific expression of an inserted transgene. To do this, we introduced a transgene cassette into two different chromosomal locations in mice: the apoAI-CIII locus on chromosome 9 and the Hprt locus on the X chromosome. The targeting cassette was composed of a neomycin resistance (Neo) gene driven by the herpes simplex thymidine kinase promoter with polyoma enhancers (9) and a human haptoglobin (Hp) gene driven by its own 1.1-kb promoter sequence (10); haptoglobin is the major hemoglobin-binding protein in serum, and its gene is mainly expressed in the liver. Our results demonstrate that the specificities of the two chromosomal regions and

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1 The abbreviations used are: ES cells, embryonic stem cells; apo, apolipoprotein; Gapdh, glyceraldehyde-3-phosphate dehydrogenase gene; Hp, human haptoglobin gene; Hp, Hp 2 allele; hHp, Hp 2 transgene; mHp, mouse haptoglobin gene; Hprt, hypoxanthine phosphoribosyltransferase gene; LPS, lipopolysaccharide; Neo, neomycin resistance gene; kb, kilobase(s); bp, base pair(s); PIPES, 1,4-piperazineethanesulfonic acid; RT-PCR, reverse transcription-polymerase chain reaction.

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of the two promoters are preserved, but their interactions are not specific.

**EXPERIMENTAL PROCEDURES**

The Neo-hHp2 Transgene Cassette—The entire human Hp2 gene (10) is contained within a 9-kb fragment extending from an XbaI site at approximately 1.1 kb 5′ to the starting codon to an ApaI site 1.6 kb 3′ to the stop codon. This fragment with 1.1-kb DNA encoding the pMC1neoPoly(A) gene (Stratagene) attached to its 5′ end in the opposite transcriptional orientation was used as a Neo-hHp2 transgene cassette.

Gene Targeting—The vector to introduce the Neo-hHp2 cassette into the Hprt locus was a modified form of the previously described locus-specific targeting vector, pMP8 (14). The regions of homology are 4 kb of DNA 5′ to the Hprt locus and 1.6 kb of DNA extending 3′ from a HindIII site in intron 2 to an EcoRI site in intron 3 of the Hprt gene. The Neo gene and the hHp2 gene were inserted as shown in Fig. 1. A 9-kb BamHI fragment of mouse genomic DNA containing the Apo-I gene and a 3′ part of the Apo-3 gene (7) was used to make the targeting construct designed to insert the Neo-hHp2 cassette into the apoAI-CHI region. The Neo-hHp2 cassette was inserted so as to replace a 450-bp BglII-BstXI fragment 3′ to the Apo-3 gene. The BstXI site is at 30 bp downstream of the poly(A) addition signals for the Apo-3 transcripts. A herpes simplex thymidine kinase gene was placed at the 3′ end of the construct (2). Cell culture, electroporation and selection using mouse ES cells (BK4, a subclone of E14T2G2a, provided by Dr. B. Koller, University of North Carolina) were as described (7) Microinjection of ES cells and derivation of transgenic mice were as described (15). Mice were maintained according to NIH/Institutional guidelines.

Southern Blot Analyses—Southern blots were made with HindIII digests of genomic DNA isolated from cells or from tails of mice at weaning (3 μg). The validity of the assay was established by the linearity of the signal for the Hp and the Gapdh genes over a range of total liver RNA from 0 to 40 μg and with primer amount varying from 0 to 105 cpn. Because the haptoglobin primer hybridizes to both human and mouse transcripts equally well, the relative amounts of transcripts present in a sample can be readily compared. For comparisons between samples or between different experiments, the expression levels were adjusted against that of Gapdh with the caution that the normalized values in different tissues may not be completely comparable, because expression of Gapdh itself is somewhat variable in different tissues (20).

Reverse Transcription-Polymerase Chain Reaction Analyses (RT-PCR)—Total RNA (1 μg) was treated with DNase I and used in a reverse transcription-polymerase chain reaction (RT-PCR) using a standard protocol (21). The primers specific to human Hp2 were 5′-AAGGGAGATGGAGTATACACC-3′ and 5′-TTAAGTGTTGATACCTCCA-TCTCC-3′ (10) and specific to mouse Hp were 5′-CAGGCCCCAGGAA-GAAAACCT-3′ and 5′-TACACAGGAGCTTTGAAAC-3′ (18). After initial incubation at 95 °C for 2 min, PCR for 35 cycles was at 60 °C for 1 min, 72 C for 1 min, 94 °C for 30 s, followed finally by 72 °C for 4 min. Nuclear Run-on—Isolation of liver nuclei and in vivo nuclear run-on assay were carried out according to Ntambi (22). The resulting radioactive RNA was hybridized to immobilized DNA probes for apoC-III, apoA-I, Neo, and Gapdh described above.

**RESULTS**

Generating Mice with Transgenes in the Hprt and ApoAI-CHI Locus—The two genes chosen as transgenes for the present study are the human haptoglobin transgene (hHp2) on a 9-kb fragment of human genomic DNA that includes the hHp2 gene with its own promoter and 3′ untranslated sequence and neo-mycin-resistant gene (Neo) from the pMC1neoPoly(A). The two genes are in opposite transcriptional orientations.

The overall scheme for inserting the Neo-hHp2 transgene cassette into the partially deleted Hprt locus on the X chromosome of BK4 ES cells is diagrammed in Fig. 1. The targeting vector is derived from pMP8SBK (14) used in our previous studies of targeted transgenesis at this locus. In the current vector, the promoter and exons 1 and 2 of the Hprt gene in pMP8SBK were removed, and selection to facilitate identification of targeted cells was with G418 instead of with hygromycin-B and kanamycin. One of the neo-mycin-resistant colonies was derived from the chimeras to their F1 female offspring. Mating of these F1 heterozygous females with C57BL/6 males was used to generate male progeny, and Southern blot analyses of their genomic DNA showed either a 7.1-kb or a 7.6-kb HindIII fragment, corresponding to the wild type or targeted Hprt locus (Fig. 1D). We designate the resulting hemizygous males having one copy of the hHp2 transgene on their X chromosomes as Hprt-Hp2 mice.

The scheme for inserting the Neo-hHp2 transgene cassette into the apoAI-CHI gene cluster on chromosome 9 is diagrammed in Fig. 2. The transgene replaces a 450-bp BglII/BstXI fragment between the Apoa-1 and Apo-3 genes. Approximately one in three colonies resistant to G418 and ganciclovir had an insertion of the transgene at the correct position as judged by the presence of Southern blot analyses of an 8-kb HindIII fragment in addition to a 12-kb band from the unmodified locus. The transgene was passed from chimeric males to approximately half of their offspring. We designate the resulting (heterozygous) mice carrying one copy of the hHp2 transgene as Apo-Hp2 mice. Males at the F2 or F3 generation were used for further experiments.

Expression of a Neomycin Resistance Transgene in the ApoAI-CHI Mice “Because the Neo gene in the Neo-hHp2 transgene cassette is driven by a powerful herpes simplex

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thymidine kinase promoter and a duplicated polyoma enhancer, the Neo transgene was expected to be expressed in most cell types regardless of the location of insertion (9). We, therefore, first determined the tissue distribution and relative levels of Neo transgene expression in the Apo-Hp2 and Hprt-Hp2 mice. The Neo transcripts were detectable by Northern blotting in the liver and small intestine of the Apo-Hp2 mice but not in the Hprt-Hp2 mice (Fig. 3). Comparison of the levels of Neo transcripts in different tissues was carried out by primer extension analyses as illustrated in Fig. 4A for the liver and 4B for the small intestine. Fig. 4C summarizes these and also presents analyses of RNA from lung, adipose tissue, bone marrow, and spleen. The results show that expression of the Neo transgene in the apoAI-CIII gene cluster differs considerably in different tissues. However, in general, the relative levels of expression in the various tissues parallel to that of the Apoa-1 and Apoc-3 genes, which are highly expressed in the liver and small intestine. Thus, relative to liver expression as 100%, the amounts of Neo transcripts per μg of total RNA in small intestine, adipose tissue, and lung were 57, 14, and 3% that of the liver. No Neo transcripts were detectable in the spleen and bone marrow.

Expression of the Neo transgene in the Hprt-Hp2 mice was detectable but low, at about 0.5% the expression of the Gapdh gene in the same tissues, except that no expression was detectable in the small intestine; the expression in the liver of the Neo gene in the Hprt locus was about 10% that in the apoAI-CIII locus.

Expression of the Human Hp2 Transgene in the Apo-Hp2 Mice—Liver is the major organ of haptoglobin production in humans and in mice. Northern blot analysis of liver total RNA (Fig. 3A) using a probe (hpβ) derived from the β region of the human Hp2 gene showed that human haptoglobin transcripts were readily detectable in the Apo-Hp2 mice. The level of expression in the transgene homozygotes with two copies of the transgene was approximately twice as much as that in the single copy heterozygotes. Human Hp2 transcripts were also detectable by Northern blot in the intestine of the Apo-Hp2 mice, but in considerably less amounts (Fig. 3B). Expression of the endogenous mouse Hp gene (detectable by a low level cross-reaction of its mRNA with the hpβ probe) was stimulated
by LPS in the liver but not in the intestine.

To compare the expression of the human \(Hp^2\) transgene with the expression of the endogenous mouse haptoglobin gene in tissues where the levels are too low for Northern blot analysis, we used primer extension analyses as illustrated in Figs. 5A through 5D and presented in a quantitative manner in Fig. 5E. The data show that the endogenous mouse \(Hp\) gene is expressed at about 15% that of the liver level in lung, adipose tissue, and bone marrow, respectively. Endogenous mouse \(Hp\) transcripts were also detected (not shown) in the thymus and

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**Fig. 3.** Northern blot analysis of the mRNA from liver (A) and small intestine (B). RNAs blots were sequentially hybridized to the six probes indicated. The haptoglobin probe was used first; it is a fragment corresponding to the \(\beta\) subunit of \(hHp^2\). Subsequent probes were specific for the genes coding for Apoa-1, Apoc-3, Apoa-4, Neo, and Gapdh. RNAs were collected without treatment (LPS \(-\)) or after LPS injection (LPS \(+\)). Each lane contained 15 \(\mu\)g of total RNA. WT, wild type.

**Fig. 4.** Primer extension analysis of expression of the Neo transgene. mRNAs (20 \(\mu\)g) from the liver (A) and from the small intestine (B) of the indicated animals were subjected to primer extension analyses with Neo primer (\(10^5\) cpm) and with GAPDH primer (\(10^5\) cpm). LPS \(+\) or LPS \(-\) indicate RNA from 12 h after LPS injection or from untreated mice. C, densitometric analyses of the Neo gene expression in various tissues from the Apo-Hp2 and the Hprt-Hp2 mice. The values are means of three mice S.E. and are expressed relative to the signals of Gapdh in the liver as 100. LI, LU, AD, BM, SP, and SI are liver, lung, adipose tissue, bone marrow, spleen, and small intestine. WT, wild type.
adrenal gland at approximately 5% that of the liver, with still lesser amounts in the intestine, spleen, kidney, and testis.

Increased expression of the mouse gene was observed in the liver after LPS treatment, which is known to induce synthesis of haptoglobin, an acute-phase response protein. The amount of human \( Hp^2 \) transcripts in the liver of Apo-Hp2 mice from a single copy of the transgene was approximately 70% that of a single copy of the endogenous mouse \( Hp \) gene. The mRNA levels of both the endogenous mouse \( Hp \) gene and the \( hHp^2 \) transgene were increased equally by about 30% at 12 h after LPS treatment. Human \( Hp^2 \) transcripts were also found in the small intestine, lung, adipose tissue, bone marrows, and spleen, at about 3% of the liver expression of the endogenous mouse gene. Less than 1% of liver levels of transcript were seen in the other tissues examined (not shown), including brain, thymus, heart, kidney, adrenal gland, testis, and ovary. In general, expression of the human transgene in the Apo-Hp2 mice in these extra-hepatic tissues was approximately half or less of the endogenous mouse gene expression, except that in the intestine expression of the transgene, which was approxi-
mately five times that of the mouse gene (itself very low at approximately 1.5% of the liver expression).

Expression of the hHp2 Transgene in the Hprt-Hp2 Mice—In the Hprt-Hp2 mice, the level and tissue distribution of transgene expression was markedly different from that in the Apo-Hp2 mice. No transcripts of the hHp2 transgene were detectable by primer extension analyses in the liver, adipose tissue, small intestine, and kidney in the Hprt-Hp2 mice, although they were detectable when total RNA was subjected to RT-PCR analyses (data not shown). Transcripts, detectable by primer extension analyses in the lung, bone marrow, and spleen, were, respectively, at about 4, 3, and 2% levels of the endogenous mouse gene in the liver. Expression in the brain, heart, and testis was detectable but at less than 1% of the liver expression (data not shown). LPS treatment did not affect expression of the transgene in any of these tissues.

Influence of the Neo-hHp 2 Transgenes on the Expression of Nearby Apolipoprotein Genes—To investigate whether the insertion of the transgene cassette approximately 30 bp 3’ to the Apoc-3 gene had influenced the expression of the nearby Apoa-1, Apoc-3, and Apoa-4 genes, we used Northern blots for Fig. 3. Sequential hybridization experiments were performed using probes corresponding to each of these apolipoprotein genes. The amounts of Apoa-1 and Apoa-4 transcripts in the Apo-Hp2 mice were different from those in the wild type mice. However, the amount of Apoc-3 transcripts was reduced to about 50% that of wild type in homozygous Apo-Hp2 mice. Thus the insertion of the transgene cassette approximately 30 bp 3’ to the poly(A) addition signal sequence affected the Apoc-3 gene expression, although the transcriptional orientations of the Neo genes and the Apoc-3 genes are opposed (Fig. 2). A preliminary nuclear run-on experiment with the liver of a homozygous Apo-Hp2 mouse showed that initiation of the Apoc-3 gene transcription appears to be normal (data not shown). This suggests that the likely cause of the undetectable amounts of Apoc-3 gene mRNA in the Apo-Hp2 homozygotes is that insertion of the transgenes just downstream of the gene results in an unstable message or in failure of the transcripts to be processed properly. The levels of expression of the Apoa-1, Apoc-3, Apoa-4, and the Neo genes were unaltered by LPS treatment.

Ready Detection of Human Haptoglobin in the Plasma of the Apo-Hp2 Mice but Not in the Hprt-Hp2 Mice—We compared the levels of the endogenous and human haptoglobin proteins in the circulation of the transgenic mice by non-denaturing polyacrylamide gel electrophoresis of plasma proteins at pH 8.9 (Fig. 6). The amount of endogenous mouse haptoglobin differs in the plasma of different individuals, with no protein being detected in some mice. Plasma haptoglobin levels are much more variable than the corresponding mRNA levels of the animals. Reliable detection of haptoglobin in the plasma of all mice was, however, achieved by treating them with LPS, which induces the synthesis of haptoglobin. Mouse haptoglobin, like the monomeric form of human haptoglobin, hp1, contains one free sulfydryl group in its α subunit and migrates as a single tetrameric (αβ₂) band, like human haptoglobin type 1-1 individuals. The human hp2 protein α subunit, in contrast, contains two free sulfydryl groups and forms a series of heteromultimers in the presence of hp1 proteins. Thus, in human type 2-1 heterozygotes, haptoglobin migrates as a ladder during gel electrophoresis. A similar ladder of haptoglobin heteromultimers, detectable in the Apo-Hp2 mice after LPS treatment, demonstrates that the haptoglobin hp2 protein is secreted in combination with the type 1-like mouse haptoglobin. Human haptoglobin was readily detected in the plasma of (homozygous) Apo-Hp2 mice having two copies of the transgene, even without LPS treatment. Plasma from human type 2-1 heterozygous individuals yields a ladder with up to 9 identifiable bands. But only three bands are detected in the plasma of Apo-Hp2 mice, with the first being the heaviest. This pattern is very similar to that seen in individuals having the haptoglobin 2-1-modified phenotype (23), which is caused by the production of less hp2 protein than hp1 (16). Polyacrylamide gel electrophoresis analysis of plasma from the Apo-Hp2 mice, therefore, demonstrates that the human haptoglobin is made and secreted in these mice, but the amount of the human hp2 protein is less than the mouse protein, consistent with the mRNA data presented above, showing that the expression of the Hp2 transgene is somewhat lower than that of the endogenous mouse gene.

In marked contrast to these observations with the Apo-Hp2 mice, no human haptoglobin was detectable in the plasma of Hprt-Hp2 mice, even after LPS-treatment, although the endogenous mouse haptoglobin was normally present.

DISCUSSION

Our results show that the chromosomal location of a transgene markedly and with a considerable degree of predictability affects the levels of its expression. Thus the Neo gene in the Hprt locus is expressed in many tissues of the Hprt-Hp2 mice at a uniform level, reflecting the ubiquitous pattern of the Hprt gene expression. However, although the Neo gene is driven by a strong viral promoter, its expression in the Hprt locus is relatively low. In contrast, the tissue expression of the same
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The apoAI-CIII region of the Apo-Hp2 mice takes on the same strong expression pattern as the apolipoprotein locus, which is highly expressed in the liver and intestine. Expression in the liver of the Neo gene in the apoAI-CIII locus is at least 9-fold that of the same gene in the Hprt locus.

In contrast to the Neo gene with its viral promoter, the liver expression in the Hprt-Hp2 mice of the hHp2 transgene driven by its own promoter sequence was only detectable by RT-PCR. This agrees with earlier work in which a sequence that included the 1.1-kilobase pair promoter of hHp2 gene was not able to support high levels of liver expression of reporter genes randomly inserted into the genome as transgenes (12, 13), despite its being sufficient for liver-specific expression of the gene in hepatic cells in tissue culture (11). Thus, high levels of liver expression of the haptoglobin gene require not only a liver-competent promoter but also enhancer element(s) outside of the 9-kb DNA containing the hHp2 gene. The apoAI-CIII region, but not the Hprt locus, can supply the enhancer element(s), and the hHp2 transgene inserted close to the Apoa-1 gene is expressed in the liver at approximately 70% of that of the endogenous mouse Hp gene. The apoAI-CIII region is also known to contain enhancers that functions in the intestine, and the expression of the hHp2 transgene in this tissue in the apoAI-CIII locus was five times that of the endogenous mouse Hp gene in the same tissue. These results demonstrate that tissue-specific enhancer elements present in the apoAI-CIII region can interact with the hHp2 promoter as well as with the viral thymidine kinase promoter and that high levels of expression of the transgene can be obtained in the liver by interactions between either of these quite different promoters when they are inserted into the genome within the range of the tissue-specific enhancers.

Studies using transgenic mice have shown that a 256-bp sequence from the human apoA-1 gene (24) or a 780-bp sequence from the human apoC-III gene (25) are sufficient for driving their respective gene expression at high levels in the liver. The cis-elements within these regions that are necessary for the liver-specific expression have been well characterized (26). The same studies also showed that neither element is able to support the expression of the transgenes in the intestine, but that high levels of intestinal expression of the transgenes can be achieved by the simultaneous presence of a DNA segment 5' to the apoC-III gene (~500 to ~900) that functions as a classical enhancer. This distal DNA segment, which contains multiple regulatory elements, also enhances expression from the apoA-I and apoC-III promoters in the liver 5-10-fold in the tissue culture system (24). The enhancement of expression in both the liver and the intestine of the Neo-hHp2 transgenes when inserted into the mouse apoAI-CIII region is most likely the consequence of similar interactions.

Unusual aberrant expression of a foreign gene in a particular transgenic mouse line is frequently attributed to chromosomal position effect, although proving this effect is often difficult. In one such case of an aberrant tissue expression of a transgene, Al-Shawi et al. (27) demonstrated that the expression patterns of the transgene changed by recovering and reintroducing it into the secondary transgenic mice. Position independence of transgene expression can be obtained when relatively large genomic segments are introduced or when the transgene is attached to a sequence for matrix attachment sites (or scaffold attachment sites) (28) or to very strong enhancers such as the locus-controlling region of the β-globin genes (29). Our work clearly demonstrates how expression levels in various tissues is affected by interaction between the promoter elements of the introduced transgene and enhancers that reside near the location of the transgene. It will be of interest to test how the introduction of matrix attachment sites or a locus-controlling region will influence the expression patterns of endogenous genes near a chosen chromosomal insertion site.

The predictable expression of the Neo and hHp2 transgene in the liver of the Apo-Hp2 mice demonstrates the usefulness of the apoAI-CIII region for introducing a targeted transgene when expression in the liver is desirable. The transcriptional properties of the foreign promoters appear to be maintained, but their promoter activities in the liver and intestine (and potentially in the adipose tissue) are greatly enhanced. Thus the acute phase response of the Hp promoter was retained, as shown by the increase in the hHp2 gene expression, under LPS stimulation to approximately the same extent as the increase in endogenous gene expression. Inactivation of the Apo-3 gene by an insertion in opposite transcriptional orientation of the Neo gene at approximately 30 bp 3' to the poly(A) addition signal sequence was a slight complication in the present experiments, and adjustment of the position of the insertion may be desirable to eliminate or reduce the influence of the transgene on expression of the Apo-3 gene. However, the reduction of apoCIII expression itself is unlikely to influence the phenotype ascribable to the transgene, because mice completely lacking apoCIII are healthy and exhibit only a mild hypotriglyceridemia (30).

In conclusion, the ability to control the levels of tissue-specific expression of a transgene in vivo by choosing both the promoter driving the gene and the site of chromosomal integration with its nearby enhancers makes targeted transgenesis a very versatile approach for achieving a predictable in vivo function of the transgene. Furthermore, by using repeated insertion of variant transgenes into the same locus, targeted transgenesis allows the systematic dissection of in vivo promoter functions or effects of subtle structural changes in the products of the transgenes.

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