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Genetics

Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants

(bacterial restriction/DNA methylation/cloning mammalian DNA/heterogeneous transgene expression/insulin gene regulation)

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ABSTRACT **Plasmids comprising transgene insertions in four lines of transgenic mice have been retrieved by plasmid rescue into a set of *Escherichia coli* strains with mutations in different members of the methylation-dependent restriction system (MDRS). Statistical analysis of plasmid rescue frequencies has revealed that the MDRS loci detect differential modifications of the transgene insertions among mouse lines that show distinctive patterns of transgene expression. Plasmids in mice that express hybrid insulin transgenes during development can be readily cloned into *E. coli* strains carrying mutations in two of the MDRS loci, *mcrA* and *mcrB*. In mice in which transgene expression is inappropriately delayed into adulthood, plasmids can only be cloned into *E. coli* that carry mutations in all known MDRS activities. Differential cloning frequencies in the presence or absence of the various methylation-dependent restriction genes represent a further way to distinguish regions of mammalian chromosomes. These multiply deficient *E. coli* strains will also facilitate the molecular cloning of modified chromosomal DNA.**

In some situations it has proved desirable to retrieve DNA following its transfer into cells and organisms, either as a means to isolate that DNA after functional selection or to access the chromosomal DNA adjacent to the site into which it integrated. One retrieval strategy is plasmid rescue, whereby cyclized fragments of a cellular genome that includes integrated bacterial plasmid DNA are used to transform *Escherichia coli*. Although plasmid rescue has been readily applied in yeast and flies (1–5), the frequencies of rescuing plasmids from mammalian cells have been dramatically below expectations (6–8). Since those rare plasmids that have been rescued into *E. coli* will subsequently retransform *E. coli* with normal frequencies, one can surmise that cis-acting DNA modifications interfere with plasmid rescue, and these can be removed by replication in *E. coli*. One prevalent modification of mammalian DNA that is absent from yeast and fly DNA and thus potentially involved in this restriction is cytosine methylation (reviewed in ref. 9). Recently, a series of genetic loci in *E. coli* have been identified by their ability to genetically restrict methylated DNAs (10–13). These loci are collectively labeled as methylation-dependent restriction systems (MDRS) (14). The MDRS loci currently include three that restrict cytosine methylated DNA, *mcrA*, *mcrB*, and *mcrC* (for modified cytosine restriction) (10, 12, 15), and one that restricts DNA methylated at adenosines, called *mrr* (11). In light of the existence of these MDRSs we sought to reexamine the prospects for plasmid rescue from mammalian cells in the context of *E. coli* strains carrying various combinations of mutations in the MDRS.

We report here that mutations in the MDRS dramatically improve the efficiency of plasmid rescue from four transgenic mouse lineages that each carry a hybrid gene composed of insulin gene regulatory sequences controlling expression of simian virus 40 (SV40) large tumor (T) antigen (16–18). Mice in the RIP1-Tag2 and RIR-Tag2 lines first express the large T oncoprotein at embryonic day 10 (19) and continue expression in all of the insulin-producing pancreatic β cells subsequently, which results in tumor formation between 12 and 24 weeks. In contrast, mice in the RIP1-Tag3 and RIP1-Tag4 lines show a marked delay in onset of transgene expression, which begins in the β cells at 10–12 weeks of age (20, 21) and induces tumors between 30 and 60 weeks.

MATERIALS AND METHODS

Construction of *E. coli* Strains. DH5 was derived from DH1 through the introduction of the *deoR* mutation, as will be described elsewhere (D.H., unpublished results). DH5α was constructed by sequential introduction of the Δ(*lacZYA–argF*)U169 deletion from strain SH210 (22) and the alpha complementation allele, ϕ80d*lacZ*ΔM15, from strain TB1 (23–25). MC1061 was modified by the introduction of the following mutations: *recA1* (from strain DH1), *endA1* (from strain MM294), and the alpha complementation allele (from strain TB1) to produce strain DH10. The absence of the *endA* endonuclease was confirmed by the procedure of Wright (26), and *recA* was scored by growth in 8 μg of nitrofurantoin per ml (27). DH10 also contains the *deoR* mutation (D.H., unpublished results).

The MDRS mutations were introduced into DH5α as follows. *mcrA* (28) and *mcrB* (10) were transferred from strain MC1061 via linkage to Tn*10* transposons (MC1061 and Tn*10* strains were obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, CT). The *mcrA* and *mcrB* mutations were scored by transformation with plasmid pACYC184 methylated *in vitro* with *Hpa* II methylase or *Hha* I methylase, respectively (10). DH5αMCR was isolated by generating deletions in the *hsd* region in a DH5α *mcrA mcrB* derivative containing the zjj202::Tn*10* transposon. Tetracycline-sensitive colonies were isolated from this strain using fusaric acid (29) and screened for the *mrr* phenotype with a λvir lysate grown on *E. coli* containing the cloned *Pst* I restriction enzyme and methylase (11). Deletion of the *mcrB* gene in strain DH5αMCR was verified using the procedure of Southern (30) with a probe plasmid, pUC9-13d, kindly provided by H. D. Braymer (see ref. 31). DH10 carries mutations in *mcrA* and *mcrB* by virtue of its lineage. DH10B was derived from DH10 essentially as for DH5αMCR. Strain construction in the *recA* backgrounds was accomplished by

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Abbreviations: MDRS, methylation-dependent restriction system; SV40, simian virus 40.

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Table 1. *E. coli* strains used for plasmid rescue

Strain	Genotype
DH5α	F⁻ *endA1 hsdR17* (r_k^-, m_k^+) *supE44 thi-1* λ⁻ *recA1 gyrA96 relA1 deoR* Δ(*lacZYA–argF*)-U169 φ80d*lacZ*ΔM15
DH5αm2	F⁻ *endA1 hsdR17* (r_k^-, m_k^+) *supE44 thi-1* λ⁻ *recA1 gyrA96 relA1 deoR* Δ(*lacZYA–argF*)U169 φ80d*lacZ*ΔM15 *mcrA*
DH5αm3	F⁻ *endA1 hsdR17* (r_k^-, m_k^+) *supE44 thi-1* λ⁻ *recA1 gyrA96 relA1 deoR* Δ(*lacZYA–argF*)U169 φ80d*lacZ*ΔM15 zjj202::Tn*10 mcrB*
DH5αm4	F⁻ *endA1 hsdR17* (r_k^-, m_k^+) *supE44 thi-1* λ⁻ *recA1 gyrA96 relA1 deoR* Δ(*lacZYA–argF*)U169 φ80d*lacZ*ΔM15 *mcrA mcrB*
DH5αMCR	F⁻ *endA1 supE44 thi-1* λ⁻ *recA1 gyrA96 relA1 deoR* Δ(*lacZYA–argF*)U169 φ80d*lacZ*ΔM15 *mcrA* Δ(*mrr hsdRMS mcrBC*)
DH10	F⁻ *araD139* Δ(*ara, leu*)7697 Δ*lacX74 galU galK hsdR hsdM*⁺ *rpsL deoR* φ80d*lacZ*ΔM15 *endA1 nupG recA1 mcrA mcrB*
DH10B	F⁻ *araD139* Δ(*ara, leu*)7697 Δ*lacX74 galU galK rpsL deoR* φ80d*lacZ*ΔM15 *endA1 nupG recA1 mcrA* Δ(*mrr hsdRMS mcrBC*)

introducing a pBR322 *recA*⁺ plasmid (kindly provided by R. Brent, Massachusetts General Hospital), performing the transduction procedure, and then curing the strain of the pBR322 *recA*⁺ plasmid using coumermycin (25).

Plasmid Rescue Procedure. After *Eco*RI restriction, genomic DNA was ligated at 10 μg/ml to favor circularization. Ligation efficiency was determined electrophoretically, where in the presence of ethidium bromide, closed circular DNA migrates predominantly as a high molecular weight form, above limiting mobility for linear DNA. Fifty nanograms of ligated DNA was transformed into frozen competent *E. coli* as described (32) and plated on SOB/agar plates using a carbenicillin drug selection for the plasmids (70 μg/ml). Rescued plasmids were analyzed by restriction mapping and DNA blotting, using standard techniques (33).

RESULTS

To evaluate possible effects of MDRS mutations on plasmid rescue, we constructed a series of *E. coli* strains deficient in one or more of the methylation-dependent restriction activities (Table 1). The primary series of mutations were introduced into the *E. coli* strain DH5α. One strain had mutations in *mcrA* and *mcrB* (DH5αm4); another carried a deletion extending from *mcrB* through the linked loci *mcrC* and *mrr* and, in addition, had an unlinked mutation in *mcrA* (DH5αMCR). Additional strains singly mutant in *mcrA* and *mcrB* were also generated (DH5αm2 and DH5αm3, respectively). A second strain background was also used, that of MC1061 (34), which is naturally deficient in *mcrA* and *mcrB* (35). The *recA* and *endA* mutations were introduced into MC1061 to produce a strain comparable to DH5α, which is called DH10, and the deletion from *mcrB* through *mcrC* to *mrr* was introduced into DH10 to produce a strain, DH10B, which is comparable to DH5αMCR. Transgenic mouse DNAs were digested with *Eco*RI and analyzed by plasmid rescue as outlined in Fig. 1.

Quantitative Effects of MDRS Mutations on Plasmid Rescue. Consistent with previous plasmid rescue experiments from mammalian cells, the DH5α strain, which is wild type for all MDRS loci, did not rescue significant numbers of colonies from any of the four transgenic mouse DNAs. In contrast, several of the MDRS mutants were capable of rescuing substantial numbers of clones, although there were significant quantitative differences between different mutant strains with the transgenic mouse DNAs (Fig. 2). A striking pattern was evident, in that plasmids from the developmental onset lineages (RIP1-Tag2, RIR-Tag2) rescued into most MDRS mutant strains (those containing *mcrA* and *mcrB* mutations), in contrast to the delayed-onset lineages (RIP1-Tag3, RIP1-Tag4), which could only be rescued into the multiply deficient strains (containing *mcrA, mcrB, mcrC,* and *mrr* mutations) (Fig. 2). Therefore, we conclude that a gene (or genes) localized within the deletion between *mcrB* and *mrr* in the strains DH5αMCR and DH10B must be inactivated for efficient rescue from the two delayed-onset RIP1-Tag lineages.

The requirement for MDRS mutations to achieve efficient plasmid rescue implies that the transgenic mouse DNAs are methylated. Two other sets of data support this interpretation. First, the drastic inhibition of transformation during plasmid rescue observed in wild-type DH5α is lost after the rescued plasmids have replicated in *E. coli*. Plasmids rescued

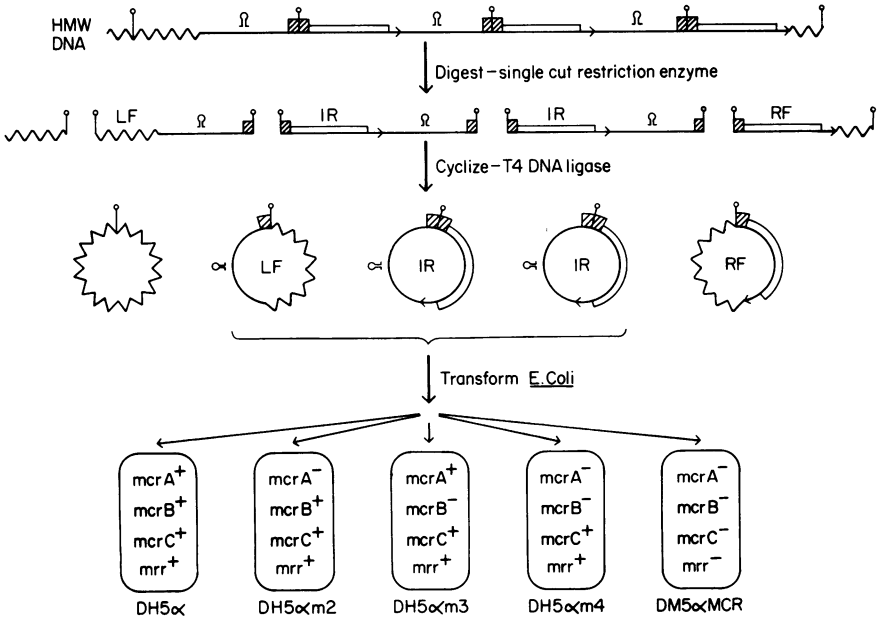

FIG. 1. Plasmid rescue scheme. Chromosomal DNA was isolated from each of the four transgenic lines, digested with *Eco*RI, and ligated to cyclize the linear molecules, thus producing left flank (LF) junctions between the chromosome and the transgene array; internal repeats (IR) of adjacent plasmids in the concatenate; and right flanks (RF), which represent the other junction. The use of *Eco*RI as the rescuing enzyme with these transgenes results in LF and IR molecules that should include functional plasmid sequences, whereas the RF from this simple concatenate would lack a plasmid origin of replication. Thus transformation of *E. coli* selects for LF and IR rescuants (bracketed). *E. coli* strains carrying mutations in the methylation-dependent restriction loci (*mcrA, mcrB, mcrC,* and *mrr*) (see Table 1) were used to rescue the transgenic plasmids from the four mouse lineages. Hatched boxes represent insulin promoter and open boxes represent the SV40 early region. ↑, *Eco*RI restriction site; Ω, plasmid origin of replication and ampicillin-resistance gene; HMW DNA, high molecular weight DNA.

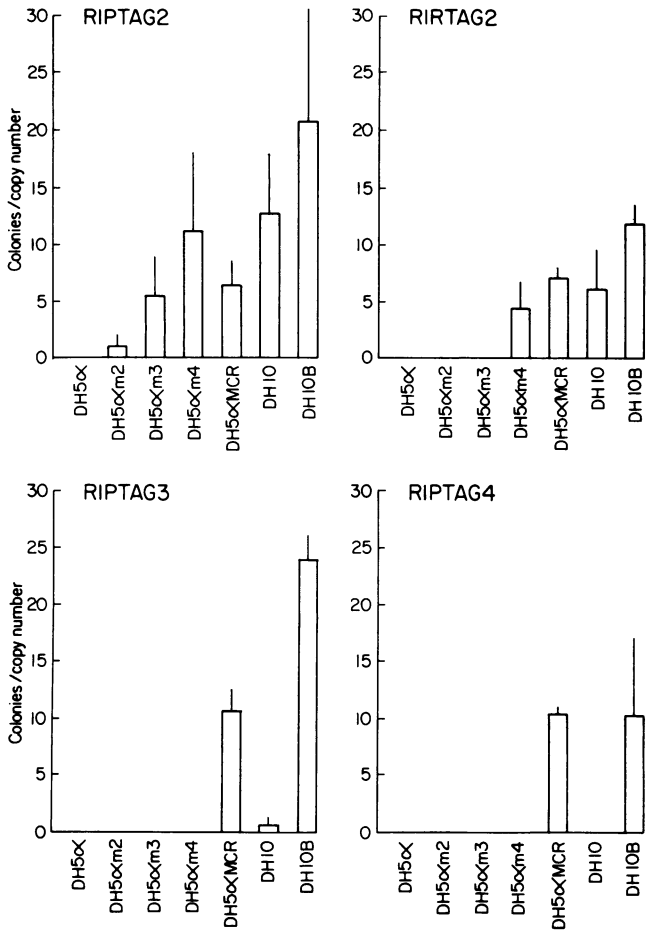

FIG. 2. Differential frequencies of plasmid rescue into MDRS mutants. The bars represent the normalized frequencies of bacterial colonies (y axis) obtained after transformation of liver DNA from four lineages of transgenic mice into wild-type and MDRS mutant strains of *E. coli* (x axis). The plasmid rescue data from the developmental-onset class of mice are presented in the top two panels (RIP1-Tag2 and RIR-Tag2), and those for the delayed-onset class are in the bottom two panels (RIP1-Tag3 and RIP1-Tag4). Quantitative comparison of plasmid rescue efficiency between the various *E. coli* MDRS stains and the four transgenic mouse lines was made after normalizing the frequencies of colonies formed for individual strain transformation efficiencies and for different transgene copy numbers among the transgenic mouse lineages; data are expressed as number of colonies rescued/10^9 transformation efficiency per transgene copy. Histograms show mean and standard error for triplicate experiments. The MDRS status of the bacterial strains is as follows: DH5α = wild type; DH5αm2 = *mcrA*; DH5αm3 = *mcrB*; DH5αm4 = *mcrA*, *mcrB*; DH5αMCR = *mcrA*, Δ(*mrr* hsdRMS *mcrBC*); DH10 = *mcrA*, *mcrB*; DH10B = *mcrA*, Δ(*mrr* hsdRMS *mcrBC*).

into any of these strains can subsequently be retransformed into DH5α and any of the other strains with comparable efficiencies, of $>10^8$ colonies per μg of DNA (not shown). Second, using restriction endonuclease digestion with pairs of methylation-sensitive and insensitive enzymes followed by Southern blotting, we found evidence for transgene methylation, although developmental and delayed-onset DNA could not be clearly distinguished (not shown).

Characterization of Rescued Plasmids and Transgene Integration Sites. Analysis of the plasmids rescued from the RIP1-Tag2 integration site exemplifies the structural analysis performed on all four lines and establishes that multiple plasmids including internal repeats and left and right flanking clones were isolated from RIP1-Tag2. Together these rescued plasmids provide a description of the integration locus (Fig. 3). This map is based upon the following data. Two of the rescued plasmids (pRIP1-Tag2.C1 and pRIP1-Tag2.C2) comigrated with single-copy bands on genomic Southern blots (Fig. 3*A*). Restriction mapping indicated that each of these plasmids contained nontransgene DNA, of about 1 kb and 1.5 kb, respectively (Fig. 4). Southern blots using probes isolated from these nontransgene sequences showed that each is derived from the same chromosomal locus of the mouse genome, yet the sequences do not hybridize with each other (Fig. 3 *B* and *C*). Restriction fragment length polymorphism analyses confirmed that the pRIP1-Tag2.C1 and pRIP1-Tag2.C2 contain junctions between the RIP1-Tag2 transgene and mouse DNA (Fig. 3 *B* and *C*). Analysis of nontransgenic mouse DNA showed that the cellular DNAs in the pRIP1-Tag2.C1 and pRIP1-Tag2.C2 flanking clones are separated by about 1.5 kb, which was deleted during transgene integration, and that the flanking DNA has not otherwise undergone any detectable rearrangement (not shown).

A similar analysis was performed on plasmids rescued from the other three transgenic insertions. In addition to the expected large number of internal-repeat clones, a number of rescued plasmids were repeatedly rescued, and each has provided information about the structure of their integrations (Fig. 4). These plasmids include (*i*) a left flanking clone (pRIR-Tag2.C2) from the insertion in the RIR-Tag2 line, which included a highly repetitive sequence; (*ii*) a junction clone (pRIR-Tag2.C1) between two adjacent integrated plasmids in this integration, one the expected RIR-Tag plasmid and the other an unexpected pBR322 molecule that was found to have contaminated the original microinjection solution; (*iii*) an internal-repeat clone (pRIP1-Tag3.C1) containing a deletion of sequences within the transgene of the RIP1-Tag3 insertion; (*iv*) head-to-head junctions between two plasmids in the RIP1-Tag3 transgenic array (pRIP1-Tag3.C2 and pRIP1-Tag3.C3); and (*v*) a junction plasmid (pRIP1-Tag4.C2) between a transgene and *E. coli* DNA that presumably contaminated the original microinjection solution and became incorporated into the RIP1-Tag4 insertion. All of these rescued plasmids were shown by DNA blotting analysis to be present in the respective integration locus and thus have served to more fully characterize those structures.

DISCUSSION

E. coli strains with mutations in the methylation-dependent restriction loci dramatically improve the efficiency of cloning plasmids integrated into the genomes of transgenic mice by means of plasmid rescue. The rescued plasmids have provided structural information about the transgenic integration sites, and statistical evaluation of plasmid rescue frequencies has revealed an interesting characteristic—namely, that the methylcytosine-dependent restriction genes detect distinct modifications of the four different chromosomal regions into which the transgenes integrated.

Cloning and Structural Analysis of Chromosomal Integration Sites. Cellular DNA representing junctions between transgene arrays and flanking chromosomal DNA have been isolated from the RIP1-Tag2 and RIR-Tag2 insertions. These cloned murine DNAs constitute markers of the particular chromosomal locations into which transgene integration occurred and can now be used for studies on the normal chromosome and the transgenic variant, as the initial analysis has demonstrated. Though the work reported here resulted in the isolation of chromosomal flanking DNAs from only two of the four lines, this is likely the result of our use of a single restriction enzyme (*Eco*RI) to release the integrated plasmids from chromosomal DNA. It should now be possible to use other restriction enzymes, as well as partial digestion with *Eco*RI, to obtain the flanks from the other two lines, given the large numbers of plasmids that can be easily retrieved using the multiply deficient MDRS strains.

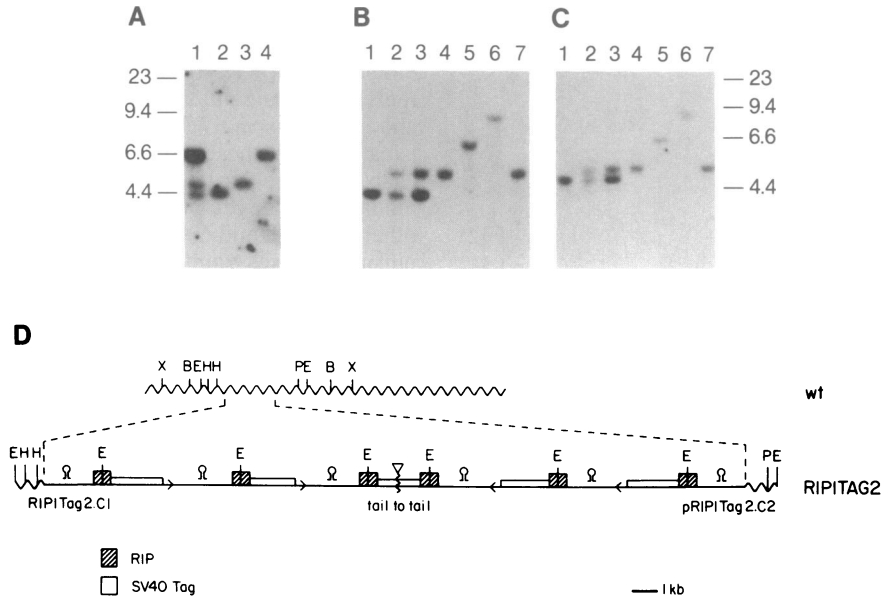

FIG. 3. Analysis of the RIP1-Tag2 chromosomal integration. (*A*) DNA blotting analysis to confirm plasmids as rescuants from RIP1-Tag2 DNA. *Eco*RI-cleaved DNAs were electrophoresed through 0.7% agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled pBR322 sequences. Lanes: 1, 20 μg of RIP1-Tag2 transgenic DNA; 2, 20 μg of control mouse DNA (C57BL/6J) doped with 20 pg of pRIP1-Tag2.C1; 3, control DNA doped with pRIP1-Tag2.C2; 4, control DNA doped with internal-repeat clone rescued from RIP1-Tag2. (*B* and *C*) Restriction fragment length polymorphism analysis of flanking DNA: Analysis of the putative flanking clones of the RIP1-Tag2 integration. (*B*) Probed with a 500-base-pair (bp) purified *Hin*dIII–*Hin*dIII flanking DNA fragment from pRIP1-Tag2.C1. The lanes (20 μg of genomic DNA) of *B* contain RIP1-Tag2 *homozygous* DNA cut with *Eco*RI (lane 1); RIP1-Tag2 *heterozygous* DNA cut with *Eco*RI (lane 2); control mouse DNA doped with 20 pg of pRIP1-Tag2.C1 cut with *Eco*RI (lane 3); control DNA cut with *Eco*RI (lane 4); control DNA cut with *Bam*HI (lane 5); control DNA cut with *Xba* I (lane 6); and control DNA doped with pRIP1-Tag2.C2 cut with *Eco*RI (lane 7). (*C*) Probed with a purified 1.5-kilobase (kb) *Eco*RI–*Bgl* I flanking DNA fragment from the second putative flanking clone rescued from RIP1-Tag2 (pRIP1-Tag2.C2). The lanes contain RIP1-Tag2 *homozygous* DNA cut with *Eco*RI (lane 1); RIP1-Tag2 *heterozygous* DNA cut with *Eco*RI (lane 2); control mouse DNA doped with 20 pg of pRIP1-Tag2.C2 cut with *Eco*RI (lane 3); control DNA cut with *Eco*RI (lane 4); control DNA cut with *Bam*HI (lane 5); control DNA cut with *Xba* I (lane 6); and control DNA doped with pRIP1-Tag2.C1 cut with *Eco*RI (lane 7). Note that the bands in lanes 4–6 of *A* and *B* are the same with both probes, and yet the two probes do not crosshybridize (lanes 7 of *B* and *C*). Sizes are indicated in kb. (*D*) Restriction map of the normal and RIP1-Tag2 chromosomal regions. The segment of the normal chromosome inferred from the rescued plasmids is shown on the top of that deduced for the transgenic integration locus. Nontransgenic DNA (wt) is aligned above RIP1-Tag2 DNA. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pvu* II; X, *Xba* I.

In previous studies, several transgenic loci have been mapped, and flanking DNAs have been isolated (36–40). In these cases, the isolation of the internal repeats and flanking clones from bacteriophage λ libraries proved laborious. Plasmid rescue into MDRS mutants has the potential to significantly improve the retrieval of DNAs flanking insertional mutations by transgenes introduced by embryo microinjection, retroviral infection, or by way of embryonic stem cells, provided that plasmid sequences are included with the transferred genes. Moreover, knowledge of the modifications inferred from the MDRS mutants will prove relevant to the isolation of mammalian genes from traditional bacteriophage and plasmid/cosmid libraries. Furthermore, it has recently been shown that the use of *E. coli* lacking these MDRS genes significantly improves the rescue of integrated bacteriophage λ genomes from transgenic mice (41).

Chromosomal Position Effects, Transgene Modification, and Genetic Restriction in *E. coli*. Comparison of the frequencies of plasmid rescue into *E. coli* strains carrying different sets of mutations in the MDRS genes has revealed a significant and reproducible pattern that appears to correlate with the phenotypes observed in the four transgenic lineages. Mutations in *mcrA* and *mcrB* were sufficient for plasmid rescue from mice of the developmental-onset class (RIP1-Tag2 and RIR-Tag2) but not from the delayed-onset class of mice (RIP1-Tag3 and RIP1-Tag4). Additional mutations in MDRS loci, mapping within the deletion carried in the strains DH5αMCR and DH10B, were necessary for efficient plasmid rescue from the delayed-onset class. This correlation of transgenic mouse lineage and *E. coli* strain was consistent with two pairs of transgenic mouse DNAs being rescued into two strains of *E. coli* with different genetic backgrounds but common MDRS mutations. The data indicate that loci encoded within the *mcrB* to *mrr* deletion in DHαMCR and DH10B are detecting and restricting DNA modifications specific to the delayed-onset class of transgenic mice.

There is precedent for differential modification of transferred DNA in studies on the characteristics of retroviruses maintained in the mouse germ line following infection of early embryos with murine leukemia virus (reviewed in ref. 42). The MOV lines of transgenic mice evidenced a clear correlation between temporal patterns of proviral expression and the extent of DNA methylation of the retroviral transgenome (43). In contrast, it does not appear that the degree of methylation is directly regulating transgene expression in the RIP-Tag transgenic mice. For if the increased transgene methylation in the delayed-onset class mice was inhibiting transgene expression, then loss of methylation might be expected to correlate with onset of gene expression. To address this possibility, a plasmid rescue analysis was performed using DNA isolated from tumors expressing the transgene in the delayed-onset mice, and the frequencies of rescue were found to be indistinguishable from those of liver DNA (not shown). This result implies that the overall methylation patterns do not necessarily govern expressibility but rather may only reflect upon the general characteristics of chromosomal domains that differentially modulate expression by other mechanisms.

The known genetic loci within the MDRS deletion in the strains DH5αMCR and DH10B are *mcrC*, *mcrB*, *hsdS*, *hsdM*, *hsdR*, and *mrr*, in linear order. Together these loci span 14 kb of DNA that maps to 98.5 min on the genetic map of *E. coli*

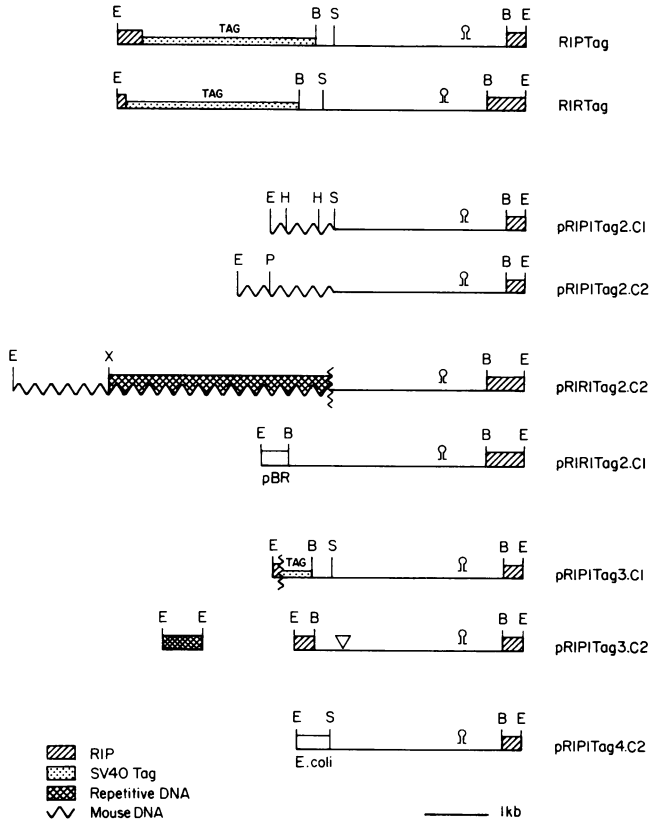

FIG. 4. Restriction maps of plasmids rescued from the transgenic mouse lines. The rescued plasmids are represented as *Eco*RI linearized constructs. The RIP1-Tag and RIR-Tag plasmids were used to generate the transgenic mice, and in each case the rescued internal-repeat clones were indistinguishable from the parental plasmids. Only flanking clones, rearrangements, and other unique structures from the integrations are shown. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pvu* II; S, *Sal* I; X, *Xba* I; ⍺, plasmid origin of replication and ampicillin-resistance gene; ▽, deletion of plasmid sequence.

K-12 (15). *mcrB* encodes a sequence-specific restriction activity, whereas the *mcrC* gene product is thought to interact with the *mcrB* gene product to increase the range of methylated target sequences that are restricted (E. Raleigh, personal communication). The *hsdR* locus encodes the type I restriction-modification system, which is mutant in DH5α and MC1061 and therefore inactive in all of the strains used in this study. The *mrr* locus restricts N^6-methyladenine-containing DNA (11). Since this modification is not known to occur in mammalian DNA (9), *mrr* is unlikely to be involved. Therefore the candidates for restriction of the delayed-onset class DNA are either *mcrC* or a new restriction locus, *mcrD*, which has recently been localized to this region (E. Raleigh, personal communication; E. Achberger, personal communication).

In conclusion, the ability to clone and concurrently assess DNA methylation of transgenes by plasmid rescue into *E. coli* strains carrying MDRS mutations is providing information about chromosomal locations that are modulating accessibility of insulin gene regulatory elements for transcription in the otherwise permissive environment of the insulin-producing β cells. The differences in transgene modification were not apparent with traditional restriction endonuclease assays and hence establish this method as a complementary tool to study aspects of DNA methylation. It can be predicted that the methylcytosine-dependent restriction genes of *E. coli* are detecting and allowing access by means of molecular cloning to a potentially important class of chromosomal regions within the murine genome.

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