Inducible Gene Knockouts in the Small Intestinal and Colonic Epithelium*

Jennifer R. Saam‡ and Jeffrey I. Gordon§
From the Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

We have developed two systems for performing Cre-mediated recombination of target genes in the rapidly self-renewing mouse small intestinal and colonic epithelium. When expression of Cre recombinase is placed directly under the control of transcriptional regulatory elements from a fatty acid-binding protein gene (Fabp), deletion of loxP flanked (floxed) DNA sequences is initiated as early as embryonic day 13.5, well before completion of intestinal morphogenesis. By embryonic day 16.5, Fabp-Cre also directs recombination in all cell layers of the transitional epithelium that lines the renal calyces and pelvis, ureters, and bladder. Fabp-Cre expression and recombination are maintained in both epithelia throughout adulthood. The second system allows recombination to be induced only in the gut and at any period during adulthood. This system uses Fabp regulatory elements to direct expression of a reverse tetracycline-regulated transactivator (rtTA). Another transgene encodes Cre under the control of tet operator sequences and a minimal promoter from human cytomegalovirus (tetO-PhCMV-Cre). In the absence of a doxycycline inducer, no basal recombination is detectable in the gut of adult tri-transgenic mice containing Fabp-rtTA, tetO-PbCMV-Cre, plus a floxed reporter gene. After 4 days of oral administration of doxycycline, recombination of the reporter is apparent in the small intestinal, cecal, and colonic epithelium. After doxycycline is withdrawn, the recombined locus persists for at least 60 days, indicating that recombination has occurred in epithelial cell progenitors that have long residency times in the proliferative units of the intestine (crypts of Lieberkuhn). This inducible system should have a number of applications for examining gene function at selected times in postnatal life, under selected physiologic or pathophysiologic conditions.

The adult mouse intestinal epithelium undergoes continuous and rapid renewal (1–6). It provides an attractive model system for examining how proliferation, differentiation, and death programs are coordinated to maintain several cell lineages. One way of auditing the contribution of specific genes to these programs is by generating null alleles using Cre recombinase expressed under the control of lineage-specific transcriptional regulatory elements. This site-specific integrase from bacteriophage P1 catalyzes recombination at 34-bp1 loxP sites that flank a gene segment to be deleted (7). A large toolbox of transcriptional regulatory elements has been assembled that function in the intestine, so it is now possible to express foreign gene products, such as Cre, in specified regions of the cephalocaudal axis of the gut, in selected cell lineages at various points in their differentiation programs, and at various stages of development (e.g. Refs. 8–12). Even with these reagents in hand, the rapid and perpetual renewal of this epithelium could pose a problem for those who wish to perform efficient and persistent conditional gene knockouts.

Epithelial cell renewal in the adult mouse intestine is fueled by multipotent stem cells located at, or near, the base of flask-shaped mucosal invaginations known as crypts of Lieberkuhn (5, 13, 14). Crypts are dynamic structures. Their average lifespan is ~110 days (15, 16). Maintenance of crypts is dependent upon their stem cell population. Once this population reaches a critical threshold and the volume of a crypt exceeds a critical size, the crypt divides (17). Division occurs by branching morphogenesis with asymmetric partitioning of epithelial progenitors among the two daughter crypts (15, 17, 18). The crypt stem cell has yet to be identified; one candidate is the crypt base columnar cell described by Cheng and Leblond (5) during lineage tracing studies that employed tritiated thymidine labeling followed by EM autoradiography.

The committed daughters of the stem cells undergo several rounds of division, forming a rapidly cycling transit cell population located in the mid-portions of each adult crypt. The stem cell ultimately gives rise to four cell types in the small intestine: enterocytes, representing >80% of all epithelial cells, goblet cells, enteroendocrine cells, and Paneth cells. The first three lineages complete their terminal differentiation during a rapid (2–5 day) and orderly migration from the crypt up an adjacent villus (1, 3, 4, 19). As mature epithelial cells approach the villus tip, they are removed by apoptosis or exfoliation. The Paneth cell lineage is the exception; it differentiates during a downward migration to the crypt base (2, 13). Paneth cells have a lifespan of ~20 days (2) and are absent from colonic crypts. The colon also lacks villi; differentiated epithelial cells emerge from a colonic crypt and form a flat hexagonal-shaped surface epithelial cuff that surrounds the crypt orifice (19).

The magnitude of epithelial cell renewal in the intestine is remarkable. On average, each small intestinal crypt produces ~300 new cells per day, a number equivalent to its steady state

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§To whom correspondence should be addressed: Dept. of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110. Tel.: 314-362-7243; Fax: 314-362-7047; E-mail: jgordon@molecol.wustl.edu.

1The abbreviations used are: bp, base pair; kb, kilobase pair; PCR, polymerase chain reaction; E, embryonic day; P, postnatal day; Fabp†1, nucleotide -596 to +21 of the rat Fabp family with four additional copies of its nucleotides -177 to -133 inserted at nucleotide -132; hGH, human growth hormone; ORF, open reading frame; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; PBS, phosphate-buffered saline; PLP, periodate-lysine-paraformaldehyde; Cy3, indocarbocyanine; rtTA, reverse tetracycline-controlled transactivator.
population. The ~7000 epithelial cells that cover a villus located in the mid-section of the intestine are replaced every ~30–40 h (e.g., Ref. 6).

These features raise several questions for those contemplating using Cre recombinase to orchestrate gene knockouts in the intestinal epithelium, as opposed to cell populations that are non-renewing and/or are long-lived. If Cre is expressed under the control of transcriptional regulatory elements that only operate in differentiating intestinal epithelial cells, will the speed of recombination be rapid enough so that the gene of interest will be silenced and its product lost before these cells are removed? How many active stem cells does a given crypt contain? Do they have equivalent mitotic rates? Do they have similar or dissimilar residence times in the crypt? If Cre is not directed to all active or potential crypt stem cells, how likely is it that within its lifespan a crypt will become populated by a mixture of cells, some with and some without the Cre-engineered recombined (null) allele?

In this report, we establish the feasibility of performing efficient Cre-mediated gene knockouts in colonic crypts. A system for inducing gene knockouts in the colonic epithelium at any time during postnatal life is described. This latter system has allowed us to explore whether a recombined null allele can persist for more than one cycle of epithelial renewal and, coincidentally, to examine the residence time of epithelial progenitors within crypts.

**EXPERIMENTAL PROCEDURES**

**Generation of Transgenic Mice**

_Fabpfk* at_ 132/Cre—This recombinant DNA consists of the following: (i) nucleotides −596 to +21 of the rat _Fabpf_ gene, with four additional tandem repeats of its nucleotides −172 to −133 added at nucleotide −132 (Ref. 11; abbreviated _Fabpfk* at_ 132) linked to (ii) a 1.8-kb fragment, containing the Cre recombinase gene with a nuclear localization signal from SV40 large T antigen (Ref. 20; a gift from Gail Martin, University of California, San Francisco), and (iii) nucleotides +3 to +2150 of the human growth hormone gene (hGH). Six pedigrees of FVB/N _Fabpfk* at_ 132/Cre mice were established.

_Fabpfk* at_ 132/loxP—lox/loxP—hGH—_pFabplXiaoZhiGH_ is a recombinant pBluescript SK− plasmid that was generated through a multistep process. It contains an insert consisting of _Fabpfk* at_ 132 linked to a 340-bp loxP site, which in turn is linked to the Escherichia coli β-galactosidase gene (lacZ). The lacZ sequence is followed by another loxP site and then nucleotides +3 to +2150 of hGH. The _Fabpfk* at_ 132/loxP—lox/loxP—hGH insert in _pFabplXiaoZhiGH_ was removed as a 6.8-kb NotI/SalI fragment and injected into FVB/N mouse embryos. The adult progeny of mice were established with this transgene._

_Fabpfk* at_ 132/rtTA—pUHG15-1-rtTA (a gift from Michael Rosenberg, Glaxo Wellcome) contains a mutant tetracycline-controlled transactivator (rtTA) (21). The rtTA open reading frame (ORF) was excised from _pUHG15-1-rtTA_ as a 1.0-kb XbaI/HindIII fragment. (Note that _XbaI_ cleaves just after the initiator ATG codon.) The nucleotide sequence upstream of the ORF was designed according to rules defined by Kozak (22), in an attempt to optimize rtTA mRNA translation. To create this sequence, two oligodeoxynucleotides (5′-GATCCACCATGT-3′ and 5′-CTAGACATGTTG-3′) were annealed to one another, and the resulting double-stranded linker was ligated to (i) the rtTA XbaI/HindIII fragment and (ii) pJS1 (pBluescript SK+—_Fabpfk* at_ 132/hGH) which had been linearized by BamHI cleavage between its _Fabpf_ and _hGH_ elements. The _Fabpfk* at_ 132 rtTA/hGH insert contained in the resulting plasmid, pColon-rtTA, was excised as a 4.0-kb EcoRI fragment and used for injections into FVB/N oocytes. Two pedigrees of FVB/N _Fabpfk* at_ 132/rtTA mice were characterized.

_tet-O_ _rpl32ol—Cre—This construct contains 7 copies of the tet operator sequence (7×_tetO_). 7×_tetO_ is located upstream of a minimal promoter from human cytomegalovirus IE1 (FlucMIV, Ref. 29), which is followed by (i) the Cre recombinase ORF, under control of the mouse_β_1-actin promoter and (ii) an intron and polyadenylation sequence from the human _β_1-actin gene. Seven pedigrees of FVB/N transgenic mice were established containing this recombined DNA.

**Inducible Gene Knockouts in the Mouse Colon**

REARING OF ANIMALS—Maintenance of Transgenic Mice—All mice used in this study were housed in microisolator cages under a strict light cycle (lights on at 0600 h and off at 1800 h). Mice were given a standard irradiated chow diet (PicoLab Rodent Chow 20, Purina Mills Inc.) ad libitum. Animals were maintained in a specified pathogen-free state. All FVB/N pedigrees were hemizygous for their transgenes. Members of each pedigree were scored by PCR. hGH DNA sequences were detected using 5′-AGGGCCCGTGGACGCGAGCG-3′ and 5′-CTGCTGGTATGTCTGACGTTG-3′ as primers. This primer pair, which spans intron 2 of the hGH gene, produces a 360-bp product. Cre DNA sequences were identified with 5′-CGGTTAAATCTCCGCGACC-3′ and 5′-CTCGCATACCGTGATCAGCAAC-3′ which generate a 149-bp PCR product. rtTA DNA sequences were detected with 5′-GCCCAGAAGCTGATTGTGTAAC-3′ and 5′-GCCCTCAGCTGGTACGTTAG-3′ which produce a 200-bp product. Cycle conditions were as follows: denaturation, 94 °C for 1 min; annealing, 55 °C for 1 min; extension, 72 °C for 1.5 min, for a total of 30 cycles.

**RNase Protection Assays—** cRNA probes for detecting mRNA transcriptions containing Cre or rtTA sequences were prepared as follows. A 400-bp BamHI/Iocl fragment from the Cre recombinase gene was subcloned into BamHI/ClaI-digested pBluescript SK+. A 232-bp StyI/SalI fragment from _rtTA_ was subcloned into SalI/VbaI-digested pBluescript SK+. In _vitro_ transcriptions were performed with XhoI-digested plasmid DNA as the template, T7 RNA polymerase, and [α-32P]UTP (NEW Life Science Products), together with reagents and protocols supplied in the MAXscript kit (Ambion, Inc.). Labeled cRNA was hybridized overnight at 50 °C to 15 μg of a total cellular tissue RNA preparation. The products were then added to reaction mixtures containing Tris-HCl (pH 7.5, final concentration 10 μM), NaCl (300 mM), EDTA (5 μM), RNase A (40 μg/ml; 100 units/mg; Sigma) and RNase T1 (2 μg/ml, 10,000 units/μg; Sigma). Following a 1-h incubation at 37 °C, RNA was extracted, precipitated with ethanol, and subjected to electrophoresis through undyed polyacrylamide gels.

A positive control was included in all RNase protection assays. A 270-bp _XhoI/Udvra_ fragment of the mouse _rpl32_ gene was subcloned into pBluescript SK+. The resulting recombiant plasmid was linearized with _ BamHI_ and _rpl32_ cRNA was synthesized using T3 RNA polymerase.

**Isolation of RNA from the Intestinal Epithelium and Mesenchyme—** The small and distal small intestine, cecum, and colon were opened along their cephhalocaudal axes, and their luminal contents were removed by washing the tissues in phosphate-buffered saline (PBS). Each segment was placed in Hanks’ buffered saline solution (HBSS) containing 25 mM HEPES and 1% calf serum (Sigma) (3 washes of 5 min each, 20 ml wash). The washed tubes were put in sterile polypropylene tubes containing 20 ml of HBSS/50 mM EDTA, and the tubes were rotated at 100 rpm for 15 min. Tissue fragments were allowed to settle, and the supernatant was removed and saved on ice. The settled tissue fragments were subjected to two more cycles of agitation in HBSS/EDTA, followed by settlement. Supernatants were pooled and spun at 250 × g for 10 min at 4 °C. RNA was isolated (RNAeasy kit, Qiagen) from the cell pellets (epithelial fraction) and the remaining tissue fragments (mesenchymal fraction) (26). Some fragments from each mesenchymal preparation were fixed in periodate/lysine/paraformaldehyde (PLP) embedded in paraffin, and 5-μm-thickness sections were cut. These sections were stained with hematoxylin and eosin and examined to verify that all the epithelium had been removed.

**Assays for Recombination**

PCR—A forward primer (HyF, 5′-GATAGAGAGGTGTAGATCGGC-3′) and two reverse primers (HyR1, 5′-CTCTACACCTACTCACTACGAG-3′ and HyR2, 5′-CTCATGCTGTCATGGGTGAGG-3′) were used to assay for recombination at the floxed hygro locus. HyF and HyR2 amplify a 680-bp fragment from the intact (unrecombined) locus. HyF and HyR1 amplify a 476-bp fragment from the recombinated locus. PCR reactions contained 100–250 ng of DNA.

Three primers were used to detect recombination of the _Fabpfk* at_ 132/loxP—loxP—loxP—hGH transgene: _Fabpf_ forward primer (flF, 5′-CTAGAGAGGTGTAGATCGGC-3′); _loxP—loxP_—hGH reverse primer (lacZR, 5′-CTCATGCTGTCATGGGTGAGG-3′); and _hGH_ reverse primer (hGHR, 5′-CTCATGCTGTCATGGGTGAGG-3′), which produce a 375-bp fragment from the intact transgene, and flF and hGHR amplify a set of two enhancers, normally located 3′ to the _H19_ gene, to be moved to a position equidistant between _H19_ and the closely linked _Igj_ gene on chromosome 7. A floxed hygromycin resistance gene (loxPHygRO2, (loxP)) was included next to the displaced enhancers when the ENHMOV allele was engineered.
a 302-bp fragment when lacZ has been removed by Cre-mediated recombination. The same thermoconditioning cycles were employed for all PCR assays of recombination (denaturation, 94 °C for 1 min; annealing, 55 °C for 1 min; extension, 72 °C for 1.5 min, for a total of 30 cycles).

Histological and Immunohistochemical Assays of Cre-mediated Recombination of Fabpl<sup>rtTA</sup>/Cre transgenic mice. Total cellular RNA was isolated from tissues harvested from a 10-week-old FVB/N transgenic mouse. The small intestine was divided into proximal, middle, and distal thirds, arbitrarily named duodenum, ileum, and colon. RNAse protection assays were performed using two labeled CRNAs: one that protects a 454-nucleotide domain from the Cre-containing mRNA transcript, and another that protects a 270-nucleotide long segment of the endogenous mouse rpl32 gene transcript.

![Image of a cell with DNA and proteins](https://example.com/image.png)

**FIG. 1.** Cre expression is limited to the distal small intestine, cecum, colon, and bladder of adult Fabpl<sup>rtTA</sup>/Cre transgenic mice.

Total cellular RNA was isolated from tissues harvested from a 10-week-old FVB/N transgenic mouse. The small intestine was divided into proximal, middle, and distal thirds, arbitrarily named duodenum, ileum, and colon. RNAse protection assays were performed using two labeled CRNAs: one that protects a 454-nucleotide domain from the Cre-containing mRNA transcript, and another that protects a 270-nucleotide long segment of the endogenous mouse rpl32 gene transcript.

**RESULTS**

**Generation of Transgenic Mice That Express Cre Recombinase in Their Distal Small Intestine, Cecum, and Colon**

We wanted to target gene knockouts to the distal small intestinal and colonic epithelium since these regions of the gut are the sites of a number of human diseases, including inflammatory bowel disease and cancer. Therefore, we chose to express Cre recombinase under the control of transcriptional regulatory elements derived from a fatty acid-binding protein gene. These elements consist of nucleotides −596 to +21 of rat Fabpl with 4 additional copies of a 35-bp sequence, spanning nucleotides −177 to −133, that had been inserted at nucleotide −132 (abbreviated Fabpl<sup>rtTA</sup> at −132) (11). Earlier light and EM immunohistochemical studies of several pedigrees of FVB/N transgenic mice had demonstrated that Fabpl<sup>rtTA</sup> at −132 can direct expression of a human growth hormone (hGH) reporter throughout the epithelium of crypts in the distal small intestine (ileum), cecum, and colon of adult mice (11).

DNA encoding Cre recombinase with a nuclear localization signal was placed between Fabpl<sup>rtTA</sup> at −132 and nucleotides +3 to +2150 of the hGH gene (encompassing all of its exons and introns). Two in-frame stop codons were positioned between the end of the Cre ORF and the initiator Met codon of hGH to prevent synthesis of hGH from the mRNA product of Fabpl<sup>rtTA</sup> at −132/Cre.

RNASex protection assays of intestinal RNAs prepared from 6–8-week-old members of six Fabpl<sup>rtTA</sup> at −132/Cre transgenic pedigrees revealed that 4 lines expressed Cre mRNA. Two pedigrees were selected for further study. Six- to 10-week-old mice from both pedigrees had indistinguishable patterns of transgene expression. Steady state levels of Cre mRNA increased from the proximal to distal thirds of their small intestine and were sustained in their cecum and colon (n = 3 mice surveyed/pedigree; see Fig. 1). Expression was also prominent in bladder and ureter but was not detectable in the renal parenchyma or in 11 other tissues (stomach, pancreas, liver, spleen, ovary or testes, muscle, heart, lung, thymus, and brain) (Fig. 1 and data not shown). This tissue-specific pattern of...
expression was maintained in 10-month-old animals (n = 2/pedigree).

Cre-mediated Recombination Is Limited to the Distal Small Intestine, Cecum, Colon, Ureters, and Bladder of Adult Bi-transgenic Mice

FVB/N Fabpl14+ at −132/Cre transgenic mice were crossed to mice with a mixed C57Bl/6-129/Sv genetic background containing a floxed hygromycin resistance gene ((loxP)hygroR(loxP)). Recombination was monitored in bi-transgenic animals using a simple PCR assay (Fig. 2A). At 6–10 weeks of age, recombination was evident in total cellular DNA isolated from jejunum, ileum, cecum, colon, ureter, and bladder and was undetectable in DNA prepared from the other tissues listed above (Fig. 2B and data not shown) (n = 6 mice). The same tissue-specific pattern of recombination was documented in 10-month-old bi-transgenic mice. Control PCR assays of DNAs prepared from the tissues of an 8-week-old bi-transgenic mouse. The 475-bp fragment is derived from the recombined hygroR locus, whereas the 680-bp fragment represents the intact locus. Note that the distance between HyF and HyR1 in the uncombined allele is >2 kb.

Defining Cellular Patterns of Cre-mediated Recombination

Characterization of a Pedigree of Transgenic Mice That Is Useful for Monitoring Cre-mediated Recombination in the Cecal, Colonic, and Bladder Epithelium—To examine the cellular basis of recombination in the adult intestine (and bladder), we generated transgenic mice containing the DNA construct shown in Fig. 3A. Fabpl14+ at −132 was placed upstream of an ORF encoding E. coli lacZ with an added nuclear localization signal. The lacZ gene was surrounded by loxP sites. Nucleotides +3 to +2150 of the human growth hormone gene (hGH) were positioned downstream of the lacZ ORF. The lacZ and hGH ORFs were separated by three in-frame stop codons. There were several reasons why we chose to use this transgene as a reporter of Cre-mediated recombination. First, at the time of our study, there were no Cre reporters that had been shown to function in the adult intestine and bladder. Second, this reporter has the advantage of identifying recombination in crypts by two criteria, loss of one foreign gene product (E. coli β-galactosidase) and gain of another (hGH). Finally, expression of these two products is controlled by the same Fabpl132 elements as those used to express Cre. We reasoned that if Fabpl14+ at −132/Cre transcription is initiated at a similar cell stratum as Fabpl14+ at −132/(loxP)/lacZ/(loxP)-hGH, then the relative distributions of lacZ and hGH along the crypt will provide some measure of the speed of Cre-mediated recombination. By pulse labeling dividing cells located in cecal and proximal colonic crypts with 5′-bromo-2-deoxyuridine, and then following their upward migration by immunohistochemical analysis of sections prepared 1 h and 1, 2, 3, 5, and 7 days later, we had determined that it takes an average of only 5 days for cells to migrate to the surface epithelial cuff and to be shed into the lumen (data not shown).

Four pedigrees of Fabpl14+ at −132/(loxP)/lacZ/(loxP)-hGH transgenic mice were generated. RNase protection assays of multiple tissue RNA samples prepared from 6- to 8-week-old adult animals indicated that the transgene was only expressed in one pedigree. Although lacZ-hGH mRNA was present in the intestine of these mice, it was limited to the cecum and colon; i.e. it was not detectable in distal small intestinal RNA.2 Southern blot analysis indicated that the transgene had inserted at a unique site in their genome and that there were −2 copies of Fabpl14+ at −132/(loxP)/lacZ/(loxP)-hGH at this site.

2 The absence of expression in the small intestine contrasts with other Fabpl14+ at −132 reporter transgenes, including Fabpl14+ at −132/Cre, and may be due to an insertion site effect, and/or to undefined cis-acting elements present in the lacZ DNA.
Fig. 3. Recombination of Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH in the distal small intestine, cecum, colon, ureters, and bladder of bi-transgenic mice. A, transgene used to monitor cellular patterns of recombination. PCR primers used to identify the recombined transgene are shown. B, PCR assay of recombination in a 12-week-old FVB/N bi-transgenic mouse containing Fabpl\(^{\text{loxP}}\) at \(-132\)/Cre and Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH. PCR products were labeled with \(^{32}\)PCTP during the course of their generation. Recombination is evident in total cellular DNA prepared from the middle and distal thirds of the small intestine (jejunum and ileum), cecum, colon, ureters, and bladder. E. coli \(\beta\)-galactosidase. X-Gal stained scattered patches of cecal and colonic crypts (Fig. 4, A and C). There was no detectable X-Gal staining of the cecal or colonic epithelium of age-matched non-transgenic littermates (data not shown), confirming that this staining was due to the product of lacZ.

Surveys of serial sections of cecal and colonic crypts present in 6–12-week-old Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH mice \((n = 4)\) revealed lacZ in epithelial cells positioned in the upper half of crypts and in their associated surface epithelial cuffs (Fig. 4E). Sensitive immunohistochemical detection methods did not reveal any hGH in the epithelium or mesenchyme (Fig. 4G).

\(E.\) coli \(\beta\)-galactosidase was also apparent in the transitional epithelium (urothelium) lining the renal calyces and pelvis and ureters and bladder of Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH mice (Fig. 5, A and C). Histochemical and immunohistochemical stains of sections prepared from the bladder disclosed lacZ throughout the urothelium (Fig. 5E) and verified that hGH was absent (Fig. 5G). X-Gal failed to stain the urothelium of age-matched nontransgenic littermates (data not shown).

\(\text{Cre}\)-mediated Recombination in the Cecal and Colonic Crypt Epithelium of Bi-transgenic mice—Mice with Fabpl\(^{\text{loxP}}\) at \(-132\)/Cre were crossed to mice with Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH. Recombination in the resulting bi-transgenic animals was assayed initially by PCR. The tissue-specific pattern of recombination mirrored the tissue-specific pattern of Cre expression, i.e., at 6–12 weeks of age, recombination of the transgene was evident in jejenum, ileum, cecum, colon, ureter, and bladder but was undetectable in stomach, renal parenchyma without urothelium, skin, muscle, brain, thymus, lung, heart, spleen, liver, pancreas, testes, or ovaries (e.g., Fig. 3). PCR assays of control, age-matched mice containing Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH alone showed no detectable recombination in any of their tissues (data not shown).

These results were confirmed by histochemical staining. Examination of wholemount preparations of cecum and colon revealed that LacZ expression was largely eliminated in bi-transgenic mice, although some patches of positive (blue) crypts remained (Fig. 4, B, D, and F). Immunohistochemical studies of cecal and colonic sections indicated that loss of lacZ was accompanied by the appearance of hGH throughout the upper half of crypts and their associated surface epithelial cuffs (Fig. 4H).

The efficiency of recombination was evaluated from two perspectives: the total number of crypts that were affected, and the extent of recombination within a given crypt. To address the first issue, we scored the number of X-Gal-positive and -negative crypts in cecal wholemounts prepared from 6-week-old Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH and bi-transgenic mice \((n = 4/group)\). Crypts were scored by taking 35-mm slide photographs of cecal wholemounts, obtained at \(\times 37.5\) magnification with a dissecting microscope, and projecting the slides onto a 1-meter-wide screen. In mice that only contained the Cre reporter transgene, the percentage of X-Gal-positive crypts was 22.3 ± 2.9 (mean ± 1 S.D.; total number of crypts surveyed = 66,686; \(n = 12,498–20,925\) crypts/mouse). In bi-transgenic mice, only 2.2 ± 1.8% of crypts were stained with X-Gal (total number of crypts surveyed = 84,009; \(n = 16,073–23,772\) crypts/mouse). The 90% reduction in X-Gal-positive crypts noted between the two groups of mice is statistically significant \((p < 0.05\) using Student’s \(t\) test).

The efficiency of recombination within each crypt was evaluated using wholemount preparations and serial sections of

Wholemount preparations of the cecum, colon, kidney, ureter, and bladder from 6- to 24-week-old Fabpl\(^{\text{loxP}}\) at \(-132\)/[loxP]/lacZ/[loxP]-hGH transgenic mice \((n = 15)\) were stained with X-Gal to detect E. coli \(\beta\)-galactosidase. X-Gal stained scattered patches of cecal and colonic crypts (Fig. 4, A and C). There was no detectable X-Gal staining of the cecal or colonic epithelium of age-matched non-transgenic littermates (data not shown), confirming that this staining was due to the product of lacZ.
cecum. As noted in the Introduction, a hexagonal-shaped surface epithelial cuff surrounds the orifice of each cecal and colonic crypt. Studies of chimeric mice have shown that each epithelial cuff is monoclonal and represents the cellular output of a single crypt (19, 27). We reasoned that if recombination occurred in some, but not all, crypt epithelial cells that support Fabp1\(^{−/−}\) at \(-132/(loxp/\text{lacZ/loxp})\)-hGH expression, we should find crypts with surface epithelial cuffs containing both lacZ-negative (recombined) and lacZ-positive (unrecombined) cells. On the other hand, if recombination were complete, then each cuff should be monophenotypic. Monophenotypic crypts were the rule rather than the exception. Surveys of wholemount preparations indicated that the percentage of surface epithelial cuffs with mixed populations of lacZ-negative and -positive cells was <0.1% in the cecums of bi-transgenic animals (n = 4 animals; 2573–3504 lacZ-positive crypts scored/mouse). Surveys of X-Gal-stained serial sections of cecum and colon also indicated that the loss of the lacZ gene product from individual crypts was complete (n = 6 mice).

The similar distribution of lacZ and hGH and the monophenotypic nature of the crypts suggest that recombination occurs over a period less than or equal to the time it takes cells to migrate from the base to the mid-portion of a crypt (~2–3 days) and/or that recombination is occurring in multipotent crypt stem cell(s), with the recombined reporter being distributed to all of its (their) progeny (see below).

**Cre-mediated Recombination Occurs in All Cell Layers of the Urothelium—lacZ expression was completely eliminated from the urothelium of bi-transgenic mice (Fig. 5, D and F). Loss of lacZ was accompanied by the appearance of hGH (Fig. 5 H).** Electron microscopic immunohistochemistry of bladders harvested from bi-transgenic animals revealed hGH throughout the urothelium, i.e. in the superficial facet (umbrella) cells (Fig. 6, A and B), in the intermediate cell layers, as well as in the basal layer (Fig. 6 C). Immunoreactive hGH was not detected by EM in the bladder epithelium of mice that contained only Fabp1\(^{−/−}\) at \(-132/(loxp/\text{lacZ/loxp})\)-hGH (e.g. Fig. 6 D).

**A System for Performing Inducible Gene Knockouts Confined to the Distal Small Intestinal, Cecal, and Colonic Epithelium**

As noted above, mice expressing Cre recombinase under the control of Fabp1\(^{−/−}\) at \(-132\) initiate recombination of a floxed locus in the intestine on or before E13.5. This early onset may be a serious limitation to investigators who wish to examine the function of a gene in the adult intestine, if loss of that gene's
product impairs or precludes normal gut development so that survival beyond birth or weaning is not possible. It would be very useful to have an inducible system that gave complete control over the timing of Cre-mediated gene inactivation, irrespective of the age of the mouse.

The inducible system we tested uses the “reverse” tetracycline-controlled transactivator (rtTA). rtTA is a chimeric protein consisting of a mutant E. coli Tn10 tetracycline resistance operon repressor linked to the acidic activating domain of herpes simplex virus VP16 (21). When the mutant rtTA binds tetracycline analogs, such as doxycycline, it acquires the ability to bind to tet operator sequences (tetO) and activate transcription of ORFs placed under the control of tetO linked to a minimal promoter (e.g., the human cytomegalovirus immediate early gene 1 (IE1) promoter (P_hCMV) (21)). Our plan was to create tri-transgenic mice that contained Fabpl43at−132/rtTA, tetOP_hCMV-Cre, and a floxed recombination test locus, where expression of Cre, and subsequent recombination of the target DNA, would be entirely dependent upon administration of doxycycline (Fig. 7A). This system for inducing Cre expression is different from a previously described system (29) that used tetR/VP16 which is only active in the absence of tetracycline (23, 30). In this latter system, Cre expression has to be suppressed by continuous administration of tetracycline until reaching the time during development or adulthood selected for induction of recombination (29).

The rtTA gene was placed upstream of nucleotide +3 of hGH in the same Fabpl43at−132/hGH vector used to generate Fabpl43at−132/Cre. As before, several in-frame stop codons were present to block production of hGH from rtTA-containing mRNA transcripts produced from this recombinant DNA. Two pedigrees of FVB/N mice with this transgene were studied. RNase protection studies of adult members of one pedigree established that the distribution of rtTA mRNA was limited to their small intestine, cecum, colon, and bladder. Unexpectedly, there was no detectable bladder expression in adult members of another Fabpl43at−132/rtTA pedigree. This latter pedigree was selected for further study because we wanted to create a system where inducible Cre-mediated recombination would be restricted to the intestine.

Fig. 7B is representative of results obtained from adult (6–40-week-old) transgenic mice from this line. The steady state
undistended bladder from a 16-week-old bi-transgenic mouse containing Fabpl at (loxP) showed no recombination at (loxP). The red arrow points to a cell in the basal layer. The boundary between the transitional epithelium and lamina propria is noted by the white dashed line. Magnification = ×63. B and C, 50–80 nm-thick sections incubated with rabbit anti-hGH and colloidal gold-conjugated goat anti-rabbit IgG, counterstained with uranyl acetate and lead, and viewed with an electron microscope. Magnification = ×13,000. B, portion of a facet cell showing broad distribution of gold particles (black dots). Note that the apical membrane is divided into rigid, concave-shaped plaques (e.g. white bracket). Plaques are linked together at interplaque hinges (black arrow), allowing folding and unfolding of the urothelial surface as bladder volume changes. The yellow arrow points to vesicles present near the apical surface. These vesicles represent sections through folds, as well as discrete discoidal cytoplasmic structures that participate in membrane recycling to and from the apical surface of facet cells. C, lower portion of a basal cell containing hGH. Arrows point to mitochondria. D, facet cells from a control mouse containing Fabpl at (loxP) (loxP)lacZ/loxP)hGH alone, stained with the same reagents as in B. There is no detectable immunoreactive hGH. Tonofilaments (TF) help maintain integrity during stretching and relaxation of the urothelium. Magnification = ×13,000. Not shown: the absence of staining in the urothelium of the bi-transgenic mouse when hGH antibodies were omitted and the sections incubated with gold-labeled secondary antibodies alone.

level of rtTA mRNA increases progressively from the proximal to distal small intestine, is maximal in the cecum, and decreases in the colon. RNase protection assays of RNAs prepared from epithelial and mesenchymal fractions of their distal small intestine (see “Experimental Procedures”) indicated that rtTA mRNA was confined to the epithelial compartment (Fig. 7, B–D). rtTA mRNA was not detected in any extra-intestinal tissues surveyed (Fig. 7B and data not shown).

Seven pedigrees of FVB/N tetO-P(r)CMV-Cre transgenic mice were analyzed next. Members of each pedigree were crossed to mice containing (loxP)hygroR(loxP). Bi-transgenic mice generated using members of two of the tetO-P(r)CMV-Cre pedigrees showed no recombination at (loxP)hygroR(loxP). Tri-transgenic mice were then produced using members of one of these tetO-P(r)CMV-Cre lines where basal Cre production is undetectable, the Fabpl at (loxP)hygroR(loxP) Cre reporter strain. 

Four- to 10-week-old tri-transgenic mice were divided into two groups. An experimental group was allowed access to water containing 2 mg/ml doxycycline and 5% sucrose for 4 days. (Oral administration of the inducer is noninvasive and allows rapid cessation of treatment.) A control group was given access to the sucrose solution minus doxycycline. (Oral administration of the inducer is noninvasive and allows rapid cessation of treatment.) A control group was given access to the sucrose solution minus doxycycline. Animals were sacrificed immediately after treatment, 4/6 after 10 days without drug, 4/4 after 20 days, and 3/5 after 30 days without doxycycline (2–3 mice/experiment; n = 2 independent experiments). Additional control experiments, using bi-transgenic mice that only contained Fabpl at (loxP)rtTA and (loxP)hygroR(loxP) or tetO-P(r)CMV-Cre and (loxP)hygroR(loxP), showed no detectable recombination after 4 days of doxycycline. Together, these controls confirmed that recombination in tri-transgenic animals reflected doxycycline-dependent rtTA transactivation of tetO-P(r)CMV-Cre.

PCR assays of colonic and cecal epithelial and mesenchymal DNAs prepared from doxycycline-treated tri-transgenic mice verified that recombination was absent from the mesenchyme (e.g. Fig. 8, B and C). This result is consistent with RNase protection studies that showed that rtTA mRNA transcripts were present in the epithelium and undetectable in the mesenchyme (Fig. 7B and data not shown).

Evidence That Recombination Occurs in Epithelial Cells with a Long Residence Time in the Crypt—Although the induction of recombination was rapid enough to occur in a time frame equal to, or less than, one cycle of renewal for each of the intestine’s epithelial cell types, we wanted to know how long the recombined allele would persist after removal of doxycycline. Therefore, groups of tri-transgenic mice were given access to water with doxycycline plus sucrose, or sucrose alone, for 4 days. Both groups were then switched to water without any supplements for an additional 10–60 days. Animals were sacrificed, and total cellular DNA was prepared from 8 tissues for PCR analysis of recombination. 0/6 tri-transgenic mice exhibited recombination when doxycycline was omitted from their drinking water. 5/6 mice manifested recombination in their intestine immediately after treatment, 4/6 after 10 days without drug, 4/4 after 20 days, and 3/5 after 30 days without doxycycline (2–3 mice/experiment; n = 2 independent experiments). The recombined allele can persist for 60 days (Fig. 8D). These
results suggest that the induced Cre-mediated recombination occurs in an epithelial progenitor cell population with a long residence time in the crypt.

To examine the cellular patterns of recombination, tri-transgenic mice containing Fabpl4 at -132/(loxP)lacZ(loxP)hGH as the Cre reporter, rather than (loxP)hygroR(loxP), were treated with doxycycline or vehicle alone, for 4 days. PCR analysis of total cellular DNA prepared from ileum, cecum, and colon established that recombination occurred only after doxycycline administration. Immunohistochemical surveys of sectioned cecal crypts from both groups of mice disclosed that recombination was associated with the appearance of hGH-positive epithelial cells throughout the upper portions of crypts and in their associated surface epithelial cuffs (Fig. 8E). These hGH-positive crypts were distributed throughout the cecum as multi-crypt patches or as isolated crypts. Since the Fabpl4 at -132/(loxP)lacZ(loxP)hGH reporter is expressed in a similar patchy fashion, we were unable to determine whether a hGH-negative cecal or colonic crypt in a doxycycline-treated tri-transgenic mouse represented a failure to induce Cre-mediated recombination in that crypt or the absence of Fabpl4 at -132/(loxP)lacZ(loxP)hGH expression. As with floxed hygroR, recombination persisted after doxycycline treatment ended. For example, Fig. 8F shows hGH-positive crypts in tri-transgenic mice that had been treated with doxycycline for 4 days followed by 10 days of water without drug.

DISCUSSION

Our studies indicate that transcriptional regulatory elements from a fatty acid-binding protein gene (Fabpl4 at -132) can be used to engineer persistent Cre-mediated recombination of a floxed target in the self-renewing adult mouse intestinal epithelium. Two systems for performing Cre-mediated recombination are described. In the first system, Cre expression is placed directly under the control of Fabpl4 at -132. Recombination in the intestine is initiated as early as E13.5, well before completion of its morphogenesis, and continues throughout adulthood. This system also permits the effects of Cre-directed recombination of a floxed allele to be evaluated in the multi-layer transitional epithelium of the renal calyces and pelvis, ureters, and bladder as well as in the simple (one cell layer thick) columnar epithelium of the intestine. The second system is more elaborate and allows recombination to be induced at any time during adulthood and only in the intestinal epithelium. The inducible system uses Fabpl4 at -132-directed expression of a reverse tetracycline-dependent transactivator (21) to control Cre expression in a progenitor cell population having a long residence time in intestinal crypts.

System 1, Fabpl4 at -132-directed Expression of Cre Recombinase in the Intestinal Epithelium and Urothelium

Recombination in the Intestinal Epithelium—This system allows constitutive expression of Cre in the intestinal epithe-
lium. We have not been able to define the precise number of Cre-expressing crypts in adult mice. This is because our Cre reporter strain of mice only support expression of the unrecombined reporter transgene in 25% of their cecal and colonic crypts. Nonetheless, our results suggest that more crypts support expression of $\text{Fabpl}^\text{4}\text{3\at 2132}$/Cre than expression of the reporter. If the fraction of Cre-expressing crypts were equivalent to the fraction that expressed the $\text{Fabpl}^\text{4\at 2132}/(\text{loxP})^\text{lacZ/(loxP)}^\text{hGH}$ reporter, then the chance that an individual crypt would express both transgenes in bi-transgenic animals would be $(0.25)(0.25) = 0.0625$, or 1 in 16, assuming that expression of each transgene is independent of the other. We found that bi-transgenic mice containing $\text{Fabpl}^\text{4\at 2132}$/Cre and $\text{Fabpl}^\text{4\at 2132}/(\text{loxP})^\text{lacZ/(loxP)}^\text{hGH}$ had a 90% reduction in the number of lacZ-positive crypts compared with age-matched $\text{Fabpl}^\text{4\at 2132}/(\text{loxP})^\text{lacZ/(loxP)}^\text{hGH}$ controls. This value is considerably greater than the 6.25% reduction we would predict by simply assuming that an equivalent fraction of crypts independently support expression of each transgene. Our analysis also indicated that, in the vast majority of cases, when lacZ is lost from a crypt due to recombination of the reporter transgene, it is lost from all of the epithelial cells of the crypt. If Cre were not directed to all active stem cells in a crypt, it would seem likely that the crypt would be populated, within its lifespan, by a mixture of cells, some with and some without the recombined DNA.

Mice containing both the $\text{Fabpl}^\text{4\at 2132}$/Cre and $\text{Fabpl}^\text{4\at 2132}/(\text{loxP})^\text{lacZ/(loxP)}^\text{hGH}$ transgenes did not lose all of their lacZ-positive cecal and colonic crypts. This indicates that a subset of their crypts does not express Cre at

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**Fig. 8. Doxycycline induction of recombination in the intestinal epithelium.**

A, induction of recombination at the $(\text{loxP})^\text{hygro}\text{R/(loxP)}^\text{locus}$ in a 6-week-old tri-transgenic mouse after 4 days of exposure to doxycycline in its drinking water. A control tri-transgenic mouse that received water without doxycycline during the same 4-day period shows no detectable recombination in any of the tissues surveyed. B, PCR assay of total cellular DNA prepared from the intact cecum and a mesenchymal (Mesen.) preparation. Recombination is not detectable in the cells that inhabit the lamina propria. C, hematoxylin and eosin-stained sections of intact proximal colon (upper panel) and a mesenchymal preparation from the same region without residual epithelial cells (lower panel). D, persistence of the recombined hygro$^\text{R}$ locus in a tri-transgenic mouse that had received doxycycline for 4 days followed by 60 days of water without any supplements. E and F, immunohistochemical analysis of the cellular patterns of doxycycline-induced rtTA-dependent recombination in tri-transgenic animals containing $\text{Fabpl}^\text{4\at 2132}/(\text{loxP})^\text{lacZ/(loxP)}^\text{hGH}$, rather than $(\text{loxP})^\text{hygro}\text{R/(loxP)}$, as the reporter. Animals were treated with doxycycline for 4 days and were then sacrificed (E) or were given water without drug for an additional 10 days (F). Sections from the cecum were stained with rabbit anti-hGH and Cy3-donkey anti-rabbit Ig. hGH-positive epithelial cells (magenta) are represented in crypts and their associated surface epithelial cuffs. Bars in C, E, and F = 25 μm.
levels sufficient to support recombination. The size of this subset will have to be defined once a Cre reporter strain is identified that supports constitutive expression of a readily scored recombination marker throughout the adult intestinal epithelium. However, the presence of a cohort of Cre-negative crypts may provide a valuable internal reference control for defining the effects of Cre-mediated gene inactivation. The intestinal ecosystem exhibits complex regional differences in the differentiation programs of its epithelial lineages, in the composition of its microflora, and in its mucosal immune system (reviewed in Ref. 31). An in vivo system for defining the effects of loss of the function a gene should, ideally, allow comparison of two juxtaposed populations of crypts, one populated by epithelial cells homozygous for the wild type allele and another populated by cells homozygous for a null allele. These juxtaposed crypts would experience a similar microenvironment in a single animal and, thus, would provide a more accurate way of defining the effects of loss-of-function than comparisons made between wild type and knockout animals. Since pathology in the human colon typically appears first in focal areas, such a system could simulate the interactions that occur at the interface between normal and abnormal cellular populations during pathogenesis. When engineering gene knockouts in the distal intestine using Fabpl4× at −132/Cre, the challenge will be to devise a system to identify this valuable subset of reference control crypts that do not support recombination.

Recombination in the Urothelium—Prior to this study, we had not appreciated that Fabpl4× at −132 was active in the urothelium. Our initial characterization of the properties of Fabpl4× at −132 in FVB/N transgenic mice had used hGH as the reporter (11). We had noted that total cellular RNA prepared from the kidneys of adult mice contained low levels of hGH mRNA (11). Since immunoreactive hGH was detectable in the proximal tubular epithelium of nephrons, we assumed that these cells were the site of hGH mRNA production (11). Based on the results obtained in the present study, this appears to have been an erroneous conclusion. The presence of hGH in proximal tubular epithelium was likely due to reabsorption of the protein from the glomerular filtrate rather than synthesis of the protein.

Fabpl4× at −132/Cre allows the effects of targeted gene inactivation to be compared in two distinct epithelia. In contrast to the gut epithelium, turnover of the urothelium is very slow: estimates in the normal adult mouse bladder range from months to over a year (32, 33). The adhesive challenges faced by the multilayer urothelium differ from the challenges faced by the intestinal epithelial monolayer. The intestinal epithelium must maintain tight cell-cell contacts to function as an effective biological barrier, yet it must also support rapid cell migration over a substrate. The urothelium must be able to stretch its surface area to accommodate marked changes in urinary volume. It must also maintain one of the most impermeable barriers in the body. The urothelium's superficial facet (umbrella) cells are able to establish and maintain this barrier, in part because they possess high resistance junctional complexes that block paracellular ion fluxes (34–36).

Fabpl4× at −132/Cre expression in the urothelium of our pedigree of transgenic mice occurs as early as E16.5. This feature will allow examination of the effects of targeted gene inactivation during critical phases of urothelial morphogenesis (37, 38). In addition, our EM immunohistochemical studies demonstrate that Fabpl4× at −132 can be used to direct Cre and reporter gene expression to all layers of the urothelium. Several studies have used nucleotides −3600 to +42 of the mouse uroplakin II gene to direct expression of foreign gene products to the urothelium of transgenic animals (39–41). Expression of these transgenes appears to be restricted to suprabasal cell layers.

System 2, Inducible Expression of Cre Recombinase in the Intestinal Epithelium

As noted above, Fabpl4× at −132/Cre expression is initiated as early as E13.5, before crypt morphogenesis begins. Therefore, Fabpl4× at −132/Cre-mediated inactivation of genes essential for intestinal development may not allow animals to survive until crypt formation is completed during the third postnatal week (42). In contrast, doxycycline-inducible, rtTA-regulated Cre expression allows a floxed target gene to be disrupted in the intestinal epithelium at any point during adulthood.

The two pedigrees of mice selected to assemble this inducible system are distinctive for the following reasons. First, adult members of the Fabpl4× at −132/rtTA pedigree only express the reverse tetracycline-controlled transactivator in their small intestinal, cecal, and colonic epithelium. Urothelial expression is undetectable in this pedigree, unlike another Fabpl4× at −132/rtTA pedigree we examined. Second, the tet-O-P2.5Cre-Cre pedigree has its transgene placed at a genomic site where it is silent in the absence of rtTA and/or doxycycline and is actively transcribed in the presence of rtTA plus doxycycline. Bi-transgenic mice generated from crosses of the two pedigrees have no discernible background of Cre expression in their gut epithelium in the absence of doxycycline and functionally significant expression when the doxycycline inducer is administered.3

Doxycycline-induced recombination of a readily scored target locus can be used to mark crypt epithelial progenitors and their progeny. The residence time of progenitors in crypts can then be inferred by noting how long the marker (recombined locus) is represented in the amplified progeny of the progenitor after doxycycline is withdrawn. By using this approach, our results indicate that recombination occurs in progenitors that are subsequently maintained in distal small intestinal, cecal, and colonic crypts for at least 60 days. This 60-day period represents over half of the estimated 110-day lifespan of a crypt (see Introduction). This finding is consistent with the results of a recent clonal analysis of intestinal epithelial progenitors that used chemical mutagenesis to mark random cells by somatic mutation of the Dlb-1 gene. The clonal analysis indicated that pluripotent crypt progenitors are retained for several months (18).

Two points need to be clarified with this inducible system. First, as with the constitutive Fabpl4× at −132/Cre expression system, we must await identification of a Cre reporter strain with robust and generalized expression in the adult gut epithelium to determine what fraction of small intestinal, cecal, and colonic crypts support recombination during and after administration of doxycycline. Second, our PCR studies indicate that the distribution of recombination in the small intestine varies somewhat between animals, with the proximal boundary ranging from the proximal third (duodenum) to the distal third (ileum). These variations cannot be correlated with the length of time that transpires between withdrawal of doxycycline and sacrifice of the mouse. We know that rtTA expression is lower in the proximal compared with the distal half of the small intestine. The variation in efficiency of recombination within

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3 The Cre ORF in the tet-O-P2.5Cre-Cre transgene is flanked by FRT sequences recognized by FLP recombinase and is positioned immediately upstream of an intron and polyadenylation signal from the human β-actin gene. Thus, the genomic locus where tet-O-P2.5Cre-Cre has inserted may be useful for rtTA-induced expression of other gene products, i.e. the Cre ORF could be excised by FLP recombinase and replaced with other open reading frames.
the proximal small intestine could be due to animal-to-animal differences in rtTA expression, the dose of doxycycline received, or to other unknown factors. At a minimum, once a better Cre reporter line is available, additional studies will have to be conducted to test the effects of different routes of doxycycline administration and different dosing schedules.

The price that must be paid for using a system where induction of recombination is achieved by ligand-regulated rtTA transactivation of Cre is more complex animal husbandry. Both Fabpl$^{+/+}$ at −132/rtTA and the tetO-P<sub>MCV</sub>-Cre will have to be introduced into mice that are homozygous for the floxed allele (or who have a floxed allele and a null allele of the gene of interest). New Cre recombinases are being described whose enzymatic activity depends upon an administered ligand (e.g. Refs. 43–46). Fabpl$^{+/+}$ at −132-directed expression of these types of Cre would simplify the task of generating mice where gene knockouts could be induced in the gut epithelium. However, it remains to be seen whether a system based on these Cre derivatives will allow a “zero” background of recombination in the absence of inducer or what the efficiency of induction will be.

Despite the complexity of the inducible system described in this report, it should facilitate definition of gene function in the intestine at selected times in pre- or postnatal life and under selected physiologic or pathophysiologic conditions.

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