High-Efficiency Type II Cell-Enhanced Green Fluorescent Protein Expression Facilitates Cellular Identification, Tracking, and Isolation

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Abstract

We have developed a transgenic mouse expressing enhanced green fluorescent protein (EGFP) in virtually all type II (TII) alveolar epithelial cells. The CBG mouse (SPC-BAC-EGFP) contains a bacterial artificial chromosome modified to express EGFP within the mouse surfactant protein (SP)-C gene 3' untranslated region. EGFP mRNA expression is limited to the lung. EGFP fluorescence is both limited to and exhibited by all cells expressing pro-SP-C; fluorescence is uniform throughout all lobes of the lung and does not change as mice age. EGFP⁺ cells also express SP-B but do not express podoplanin, a type I (TI) cell marker. CBG mice show no evidence of lung disease with aging. In 3 hours, TII cells can be isolated in >99% purity from CBG mice by FACS; the yield of $3.7 \pm 0.6 \times 10^6$ cells represents approximately 25 to 60% of the TII cells in the lung. By

FACS analysis, approximately 0.9% of TII cells are in mitosis in uninjured lungs; after bleomycin injury, 4.1% are in mitosis. Because EGFP fluorescence can be detected for >14 days in culture, at a time that SP-C mRNA expression is essentially nil, this line may be useful for tracking TII cells in culture and *in vivo*. When CBG mice are crossed to transgenic mice expressing rat podoplanin, TI and TII cells can be easily simultaneously identified and isolated. When bred to other strains of mice, EGFP expression can be used to identify TII cells without the need for immunostaining for SP-C. These mice should be useful in models of mouse pulmonary disease and in studies of TII cell biology, biochemistry, and genetics.

Keywords: alveolar type II cells; surfactant protein C; bacterial artificial chromosome; alveolar epithelium; enhanced green fluorescent protein

The alveolar epithelium, which covers more than 99% of the internal surface area of the lung (1), is comprised of alveolar epithelial type I (TI) and type II (TII) cells. The distinctive morphologic features of both cell types were initially described by transmission electron microscopy. At the light microscopic level, cellular identification has depended on biochemical and molecular markers of varying degrees of specificity (e.g., TII cells: surfactant protein C [2], ABCA3 [3], and RTII-70 [4]; TI cells: podoplanin [5, 6] and aquaporin 5 [7]).

One strategy to dissect the roles of specific cell types in development and disease is to label cells with marker proteins expressed from transgenes that contain cell-specific promoters (8). Position effect variegation often complicates this approach, resulting in line-to-line variation in the fraction of targeted cells expressing a transgene due to the random nature of vector integration into chromatin (9). Position-effect variegation has been important in prior studies designed to use a portion of the TII-specific surfactant protein C gene (2) to

label TII cells. In adult mice, human (3.7 kbp) and mouse (4.8 kbp) surfactant protein (SP)-C promoters drive transgene expression in TII cells (10–12), albeit with line-to-line variation in expression level, specificity to TII cells, and in the fraction of TII cells (10–72%) expressing the transgene.

Bacterial artificial chromosome (BAC) vectors permit transgenic expression from very large promoter regions that are more likely to recapitulate endogenous gene expression patterns with minimal position effect variegation (13). We have successfully

(Received in original form September 18, 2014; accepted in final form February 10, 2015)

This work was supported in part by National Institutes of Health grants HL-24075 and HL-57426.

Author Contributions: Conception and design: J.N.V., R.F.G., L.A., A.G., D.L., W.B.D., C.C., and L.G.D. Analysis and interpretation: J.N.V., R.F.G., L.A., D.L., C.C., and L.G.D. Drafting the manuscript for important intellectual content: J.N.V., R.F.G., L.A., D.L., and L.G.D.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 53, Iss 1, pp 14–21, Jul 2015

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Originally Published in Press as DOI: 10.1165/rcmb.2014-0348MA on February 18, 2015

Internet address: www.atsjournals.org

Clinical Relevance

The CBG (SPC-BAC-EGFP) mouse complements transgenic mice that express enhanced green fluorescent protein (EGFP) from the rat podoplanin promoter specifically in alveolar epithelial type I (TI) cells within the lung. EGFP expression in both lines reflects the differentiated state of the alveolar epithelial cells in the adult lung in that EGFP expression is under the regulation of, and therefore is dependent on, the expression of either the rat podoplanin or the mouse surfactant protein (SP)-C bacterial artificial chromosome transgenes. This contrasts with the permanent labeling of TI or alveolar epithelial type II (TII) cell lineages with Cre-recombinase-mediated activation of reporter gene expression. The CBG and Christmas mice should provide useful additions to the current repertoire of transgenic mice in that these mice are useful for identifying virtually all of the TI and TII cells and for isolating both of these cell types. The persistence of detectable EGFP, even after SP-C mRNA expression is essentially nil, permits tracking of TII cells for at least 2 weeks in culture and allows for tracking of cells for a limited time *in vivo* under varying biologic conditions. Finally, in crosses of CBG with other genetically defined lines, the high percentage of EGFP-labeled TII cells should prove valuable in the study of mouse models of pulmonary development, function, and disease.

used a BAC vector containing the rat podoplanin gene to express transgenes in virtually all alveolar TI cells (14).

In this report, we describe the CBG (SPC-BAC-EGFP) transgenic mouse, in which virtually all adult TII cells express strong fluorescence. Enhanced green fluorescent protein (EGFP) fluorescence is limited to the lung and, within the lung, to pro-SP-C⁺ cells; conversely, virtually all pro-SP-C⁺ cells express EGFP. The amount of SP-C mRNA in the lungs of CBG mice is very similar to that in wild-type mice. CBG mice are born normal and remain healthy as they age; they do not appear

to develop pulmonary pathology. The phenotype has been stable for more than 5 years. This line of mice has several practical uses: (1) rapid and efficient TII cell isolation yielding purities greater than 99%, (2) tracking of cells *in vitro* and *in vivo* for more than 2 weeks because the EGFP signal persists for more than 2 weeks after expression of SP-C mRNA essentially ceases, (3) cell cycle analysis, (4) identification of TII cells without staining for other cellular markers in crosses with other transgenic lines, and (5) simultaneous isolation of TI and TII cells from the same mouse.

The following features of this mouse distinguish it from other lines in which TII cells express EGFP: (1) virtually all TII cells express EGFP, minimizing the possibility that subpopulations of TII cells are being selected; (2) expression of EGFP remains unique to TII cells and does not change as the mice age; and (3) simplified, single-FACS sorting strategies can be used to isolate 25 to 60% of all of the TII cells in the lung.

Some of the results of these studies have been previously reported in abstract form (15, 16).

Materials and Methods

BACs

BAC RP23-247J9 (CHORI; BACPAC Resources Center, Oakland, CA) contains mouse SP-C and eight other protein coding genes: Epb4.9 (erythrocyte protein band 4.9, accession no. NM_013514), Rai16 (retinoic acid induced 16, accession no. AK090035), Nudt18 (nucleoside diphosphate linked moiety X-type motif 18, accession no. NM_153136), Hr (hairless, accession no. NM_021877), Reep4 (receptor accessory protein 4, accession no. NM_180588), Lgi3 (leucine-rich repeat LGI family member 3, accession no. NM_145219), BMP1 (bone morphogenetic protein 1, accession no. NM_009755), Phyhip (phytanoyl-CoA hydroxylase interacting protein, accession no. NM_145981.3), one tRNA^{ala} gene, and no known miRNA coding genes. The BAC was modified by recA-mediated recombination (17) with the shuttle vector p139B7 (details are provided in the online supplement), replacing nucleotides 636 to 733 in the 3' untranslated region of SP-C with 1.6 kbp of internal ribosome entry site (IRES)-EGFP sequence. Growth, isolation, and analysis of

BAC DNA were performed as previously described (14).

Transgenic Mice

C57Bl6 × DBA2 zygotes were injected with modified BAC DNA (2 ng/μl) and implanted into pseudopregnant C57Bl6 female mice using standard techniques (18). Three founder mice, identified from PCR reactions on tail DNA using primers for EGFP (5'-TGAAGTTCATCTGCACCACCG-3' and 5'-TGATGCCGTTCTCTGCTTGTC-3'), were bred to C57Bl6 partners, and one mouse transmitted the transgene to the offspring. The transgenic BAC copy number was estimated by comparison to wild-type DNA in duplex PCR assays for different numbers of amplification cycles using primers for SP-C (RT-PCR) and SP-A (5'-GCATTAGACGACAGAACTCCAGCC-3' and 5'-TACTGAGAGATGTGTGCTTGGTGAG-3'). All animal protocols complied with UCSF institutional guidelines.

Transgenic Mice in Which TI and TII Cells Are Specifically Labeled

To develop a mouse line that expressed different markers for TI and TII cells by which cells can easily be identified and FACS can be performed, we crossed the CBG mouse to the "line 9" mouse (14), which expresses rat podoplanin constitutively. TI cells can be identified by a monoclonal antibody that recognizes rat, but not mouse, podoplanin (5, 14).

Detailed methods describing techniques of shuttle vector construction, microscopy, isolation of TII cells in high degrees of purity, simultaneous isolation of TI and TII cells, cell cycle analysis, quantification of RNA and protein, and bleomycin lung injury are provided in the online supplement.

Results

CBG, a Mouse Line Transgenic for an SPC-BAC Modified to Express EGFP

We used an IRES-EGFP-modified mouse SP-C BAC to generate transgenic mice. One set of pronuclear injections led to three founder mice, of which one transmitted the transgene to its progeny. We refer to this line as the CBG mouse. Hemizygous CBG mice have between two and four copies of the transgenic BAC per haploid genome as determined by PCR cycle number titration on genomic DNA (data not shown).

CBG mice are normal at birth and remain healthy as they age. Lungs from fetal, neonatal, and adult CBG mice, both hemizygous and homozygous, appear normal with no obvious evidence of disease, injury, or defective development. CBG mice exhibit normal fecundity and transmit the transgene as predicted by autosomal Mendelian genetics. Starting with the original founder, a C57Bl6XDBA2 F1 female mouse, the line has been backcrossed for more than 30 generations to the C57Bl6

strain with no detectable changes in physiology or phenotype.

EGFP Expression Is TII Cell Specific in CBG Mice and Occurs in Virtually all TII Cells

The lungs of CBG mice are uniformly fluorescent (*see* Figure E1 in the online supplement); cellular EGFP expression is equal in all five lobes (Figure E2). EGFP fluorescence is specific to TII cells (Figures 1A–1C) and limited to alveoli, with no

expression in blood vessels or airways (Figures 1D and 1E).

EGFP fluorescence is restricted to TII cells. We used several criteria, both inclusive (i.e., presence of lamellar bodies [Figure 1C] and expression of SP-B and SP-C proteins [Figure 1J]) and exclusive (i.e., absence of mouse podoplanin [OTS8] protein [Figure 1I]), to correlate EGFP expression with TII cells and to estimate the fraction of TII cells expressing the transgene. We found no cells in which SP-C and EGFP expression was uncoupled (Figures 1H and 1N). Taken together, these findings suggest that EGFP in CBG lung is a valid surrogate marker for the SP-C gene.

SP-B protein can be detected in lamellar bodies of TII cells, in the alveolar lumen as a result of surfactant secretion, and in alveolar macrophages, which phagocytose the protein (19). Examples of coexpression of SP-B and EGFP in TII cells are shown in Figures 1M and 1N. To quantitate coexpression, we counted the number of EGFP⁺ cells that were also SP-B⁺. Of 1,026 EGFP⁺ cells, 1,020 (>99%) were also SP-B⁺. For the converse experiment, we identified SP-B⁺ cells, excluding from the count obvious alveolar macrophages present in the alveolar space, and then scored these cells for EGFP. Of 1,025 SP-B⁺ cells, 1,002 were also EGFP⁺ (>97%). Re-evaluation of the immunohistochemistry suggested to us that the SP-B⁺, EGFP[−] cells were probably macrophages that had phagocytosed SP-B protein.

The TII cell marker OTS-8 (mouse podoplanin) was never observed in association with EGFP⁺ cells in the normal adult CBG lung (Figure 1), although we have seen coexpression after lung injury (*see* Figure 3). High-power magnification shows a contiguous, uninterrupted delineation of the alveolar epithelium by either OTS8 in red or EGFP in green (Figures 1A and 1B). The fact that all SP-C⁺ cells expressed EGFP and that there were no apparent breaks in EGFP/mouse podoplanin immunofluorescence suggests that all TII cells were labeled.

EGFP mRNA Expression Is Lung Specific in CBG Mice

SP-C expression is considered to be specific to the lung and, within the lung, to TII cells (2, 10, 20). We examined the tissue specificity of transgene expression in CBG mice by comparing EGFP, SP-C, and

Figure 1. (A) Expression of the enhanced green fluorescent protein (EGFP) transgene is limited to alveolar epithelial type II (TII) cells in **SPC-BAC-EGFP** (CBG) mice. (A, B, and D) EGFP⁺ TII cells (green, arrows) and the apical plasma membranes of alveolar epithelial type I cells (red) labeled with anti-mouse podoplanin (OTS8) (34)/anti-hamster IgG-Alexa 594. (A) Combined red and green channels showing that the entire epithelium can be visualized; there are no discontinuities in the fluorescence. (B) Red channel shows that TII cells are not decorated with mouse podoplanin (arrows). (C) Matching phase contrast image showing lucent lamellar bodies (arrows) in TII cells. (D and E) Lower-magnification paired immunofluorescence and phase contrast images showing lack of either EGFP expression or podoplanin staining in airways and blood vessels (BV). The alveolar epithelial surface expresses either EGFP or podoplanin. (F–N) Colocalization of EGFP with surfactant protein (SP)-B and SP-C. (F–H) The distribution of pro-SP-C is similar in wild-type (F) and CBG mice (G and H). (I–K) Phase contrast (I) and fluorescence images showing colocalization of SP-C (J and K) with EGFP (K). (L–N) TII cells can be clearly identified at higher magnification by the presence of phase-contrast lamellar bodies (LB) (arrows) and colocalization with SP-B (M and N) and EGFP (N) in TII cells. AB, antibody; WT, wild type.

Figure 2. FACS isolation of EGFP⁺ cells from CBG lungs and persistence of EGFP expression in culture. (A) Scattergram of CBG mouse lung cells sorted for EGFP fluorescence, either without propidium iodide (PI) or with PI. Fraction 1 contained cells with very low levels of fluorescence; fraction 2 cells exhibited low fluorescence; cells in fraction 3 exhibited fluorescence approximately 100-fold higher than fraction 2. Scattergram of FACS sorted cells after the addition of PI, which stains dead/damaged cells, demonstrating that fraction 2 consists of dead/damaged cells. (B–G) Cytospins of cells from each of the three fractions showing paired phase contrast and fluorescence images. Fraction 1 (B, E) contained morphologically heterogeneous cells that were mostly not fluorescent. Fraction 2 (C, F) contained cell ghosts of very low fluorescence. Fraction 3 (D, G) contained uniform round cells of high

Table 1. Quantitative PCR Analysis of Surfactant Protein C and Enhanced Green Fluorescent Protein Expression in Lungs of Wild-Type Mice and CBG Mice

SP-C mRNA (Relative Fluorescence)		EGFP mRNA (Relative Fluorescence)	
WT	CBG	WT	CBG
906 ± 83 [†]	922 ± 36	0	971 ± 123

Definition of abbreviations: CBG, SP-C-BAC-EGFP; EGFP, enhanced green fluorescent protein; SP-C, surfactant protein C; WT, wild type.
[†]Values are means ± SD (n = 3). Relative amounts of mRNAs for EGFP and SP-C were determined by the standard curve method from dilutions of CBG lung cDNA, expressed as fluorescence relative to that found in CBG lungs for both genes. Results were normalized to endogenous levels of 18S ribosomal RNA. Methods are described in the online supplement.

β-actin mRNA levels in transgenic and wild-type mouse tissues by RT-PCR (Figure E3). In comparing expression among 10 different organs, EGFP mRNA was detected only in the lung.

CBG and Wild-Type Lung SP-C mRNA Levels Are Similar

EGFP protein in CBG mice is translated under control of an IRES element positioned downstream of SP-C protein coding sequences in the mRNA transcribed from the transgene. Because IRES initiator elements can be inefficient (21), this configuration could produce more transgenic SP-C than EGFP protein. Nonetheless, SP-C mRNA expression is very similar in wild-type and in CBG lungs (Table 1).

Isolation of EGFP⁺ Cells from CBG Mouse Lung by FACS

Figure 2 shows the results of FACS analysis of cells from CBG lungs. FACS-sorted lung cells were gated to remove cell debris and cell aggregates. The vast majority of cells in the CBG mouse were unlabeled. Fraction 1 contained cells of heterogeneous sizes and shapes that were either not fluorescent or exhibited weak autofluorescence; this cloud is also seen when FACS is performed on wild-type mice (data not shown). The EGFP-labeled cells in the CBG mouse fell into two distinct clouds on the scattergram that differed from each other in

fluorescence intensity by approximately 100-fold. Fraction 2 contained cell ghosts with very low-level EGFP fluorescence (Figures 2C and 2F) that can be removed by concomitantly labeling with propidium iodide (22) to stain dead/damaged cells (Figures 2A and 2B). Fraction 2 contained very little RNA by spectral analysis (data not shown).

Fraction 3 contained cells of uniform size (Figures 2D, 2G, and 2H); at higher magnifications, phase lucent lamellar bodies can be seen (data not shown). The cells were brightly fluorescent (~1% were EGFP⁺), with the overall intensity varying considerably from cell to cell but within the 10-fold range expected for fraction 3 from the scattergram. The intercellular variation in fluorescence intensity appeared to be caused by variability in nuclear fluorescence (Figure 2H). The highly uniform nature of the cells in fraction 3 reflects FACS parameters intentionally biased for purity and cell integrity over quantity and speed. A typical 2-hour sort started with a mixed population of approximately 60 × 10⁶ cells from a single pair of CBG lungs. The scatter gates eliminated 60% of the cells and the remaining 40% (24 million cells) were sorted as single cells, from which we obtained 3.7 ± 0.6 × 10⁶ cells (mean ± SD; n = 3) (~6% of the mixed cell prep). These cells have high viability (99 ± 0.5%; n = 3) by trypan blue exclusion and were 98 ±

0.6% (mean ± SD; n = 3) SP-B⁺ (Figures 2I and 2J). The cells can be cultured *in vitro* under conditions that maintain characteristics of the TII cell phenotype (23).

Cell-Cycle Analysis by FACS

TII cells are believed to act as progenitor cells in the alveolus. Cell cycle analysis by FACS revealed 0.9 ± 0.7% (mean ± SD; n = 4) of the cells to be actively mitotic, suggesting that in the normal rodent lung, only a very small proportion of TII cells is proliferating at a given time. By somewhat different methods, it appears that approximately 0.05% of TI cells proliferate in normal rodent lungs (23). In lungs 7 days after administration of bleomycin, 4.1 ± 2.1% (mean ± SD; n = 4) of the EGFP⁺ cells were in G2M, consistent with increased TII cell proliferation after injury.

Persistence of EGFP Fluorescence in TII Cells *In Vivo*

Because EGFP expression is under the control of the SP-C promoter, EGFP expression should decrease with time in cells cultured under conditions in which SP-C is no longer expressed. Although SP-C and EGFP mRNA levels decrease rapidly with time in culture (Figure 2T) after 3 days to approximately 0.01% of freshly isolated cells, protein levels of both SP-C and EGFP decline more slowly after 7 days to 10 to 20% of freshly isolated cells (Figure 2U). Visually detectable immunofluorescence persists for a surprisingly long time after SP-C and EGFP mRNA expression have fallen dramatically. TII cells cultured for up to 7 days fluoresce robustly. EGFP can still be detected after 17 days of culture (Figures 2Q–2S).

TII Cell EGFP in Bleomycin-Induced Lung Injury

Because EGFP signal persists for at least 2 weeks in culture, we tested whether the CBG mouse could be used to track cell lineage in injured lungs. After injury with bleomycin,

Figure 2. (Continued). fluorescence. (H–J) Higher-magnification views of cells in fraction 3 demonstrating that these cells exhibit EGFP fluorescence (H) and contain immunoreactive SP-B (I). (J) A merged SP-B/EGFP image is shown. EGFP⁺ cells all express SP-B. Cells from fraction 3 were placed in tissue culture and cultured on glass cover slips coated with fibronectin. (K–P) Paired phase contrast and fluorescence images of TII cells showing that, although cells flatten, attenuate, and spread with time in culture, EGFP fluorescence persists. Exposures were taken with constant exposure time, showing that fluorescence is weaker in culture as cells flatten and thin. (Q–S) Cells maintained for up to 17 days still have detectable fluorescence. In these images, camera exposure time was set on “automatic”; although fluorescence intensity decreased with time in culture, it can easily be detected. (T) Time course of mRNA expression in culture showing that over a 7-day period, mRNA levels for both SP-C and EGFP fall by approximately 104-fold. (U) Time course of protein expression showing that SP-C and EGFP protein levels fall by approximately 5-fold over a week.

Figure 3. TII cell shape changes in areas of lung injury after treatment with bleomycin, and cells colocalize with podoplanin. Paired immunofluorescence and phase contrast images showing immunostaining for OTS8 (red) and EGFP fluorescence (green). EGFP expression can be used to track changes in cell shape in injured lung after treatment with bleomycin. Four days after bleomycin instillation, TII cells appear to flatten (A–C), some more than others. (D–F) In some areas, one can detect colocalization of EGFP and OTS8 (arrows), which is not seen in uninjured mice.

the morphologic appearance of EGFP⁺ cells changes from cuboidal to one in which there is a perinuclear region with thin cytoplasmic extensions, similar to the appearance of TI cells. In some images, EGFP⁺ cells express OTS8 (mouse podoplanin), a TI cell marker (Figure 3). We have never observed EGFP⁺/podoplanin⁺ cells in uninjured lungs and believe these observations are consistent with transformation of TII cells to TI cells after injury.

The “Christmas Mouse,” a Mouse Expressing Different Transgenes in TI and TII Cells

We crossed the CBG mouse to the line 9 mouse, which expresses a rat podoplanin BAC transgene (14) in TI cells. In this mouse, the entire alveolar epithelium can be visualized by using an antibody against rat podoplanin for TI cells and EGFP expression in TII cells (Figure E4). One advantage of this mouse is that the fluorescence signal for rat podoplanin is much stronger than that for mouse podoplanin, which facilitates FACS of isolated cells. TI and TII cells can be simultaneously isolated from the same mouse by using elastase instead of dispase to digest lung tissue, albeit at a lower yield of TII cells than for the CBG mouse (2×10^5 TI cells and 5×10^5 TII cells/lung) (23), with greater than 97% and greater than 98% purity, respectively ($n = 6$). Although dispase is more effective than elastase in

dissociating lung cells, it damages TI cells. The use of a single animal for such studies may prove useful for molecular profiling of cells during development and after injury.

Discussion

In the CBG mouse, EGFP driven by the SP-C promoter is expressed specifically in virtually all TII alveolar epithelial cells. The EGFP-labeled cells can be easily identified and isolated in high yield (~ 25 –60% of the TII cells in the lung) and purity ($>99\%$) from adult mice for cell culture or biochemical analysis.

The features of this mouse that distinguish it from other lines in which TII cells express EGFP include (1) virtually all TII cells express EGFP, minimizing the possibility that subpopulations of TII cells are being selected; (2) expression of EGFP remains unique to TII cells and does not change as mice age; and (3) simplified, single-FACS sorting strategies can be used to isolate 25 to 60% of all of the TII cells in the lung.

Although we have focused on EGFP, the CBG mouse is also vicariously transgenic for nine other genes present on mouse genomic DNA included in the original RP23–247J9 BAC (BMP1, Phyhip, Epb4.9, Rai16, Nudt18, Hr, Reep4, Lgi3, and tRNA^{ala}), any of which has the potential to produce an altered phenotype in CBG animals. We have compared expression levels of the additional

transgenes in several other wild-type and CBG tissues and found no significant differences (data not shown). In the more than 5 years since inception of the line, we have observed nothing unusual about CBG mice, which develop, breathe, and reproduce in a normal fashion. Their lungs appear grossly and microscopically to be normal at birth and as the animals age. As mice age, EGFP expression continues to be found in apparently all TII cells; expression remains limited to TII cells.

Expression efficiency, the fraction of all CBG TII cells expressing EGFP, is dependent on the criteria that are used to measure it. One criterion is the percentage of SP-C⁺ cells that also express EGFP. By this criterion, all SP-C⁺ cells express EGFP and *vice versa*; therefore, the efficiency of EGFP expression approaches 100%. Supporting the conclusion that virtually all TII cells express EGFP is the observation that the fluorescence of the epithelial surface in the Christmas mouse (Figure E4) contains no apparent gaps.

Early TII cell flow cytometry used the intrinsic fluorescence of TII cells or fluorescently conjugated lipophilic dyes and lectins (24, 25). Roper and colleagues isolated highly purified TII cells from a human SP-C–EGFP transgenic mouse (12) using two successive sorts over a total of 5.5 hours to obtain 5 to 10×10^4 cells per mouse, with a purity of 95%. Only approximately 10% of the TII cells in this mouse express EGFP, raising the question of whether the isolated cells were a random sample of all TII cells or, alternatively, a subpopulation of the total (26). In the recently described H2B–GFP mouse (27), the authors used a combination of negative and positive sorting to isolate cells to greater than 90% purity, although the number of isolated cells was not stated. In the H2B–GFP line, efficiency and specificity of expression of EGFP in TII cells decrease with age. EGFP⁺ cells could be detected in bronchioles by 1 week of age; by 8 weeks, only 63% of TII cells expressed EGFP. In a 2-hour FACS procedure, we routinely isolate 3 to 4×10^6 EGFP⁺ cells/mouse with a purity greater than 99%. With a similar time investment, procedures using IgG-panning and differential cell adhesion yield approximately 5×10^6 cells/mouse, but at lower purities (90–92%) (28, 29). Adult mice have been estimated to contain approximately 6 to 15×10^6 TII cells (30, 31). Based on these figures, we are able to

isolate 25 to 60% of the TII cells in a CBG mouse lung.

The CBG mouse complements transgenic mice that express EGFP from the rat podoplanin promoter specifically in TI cells within the lung (14). EGFP expression in both lines reflects the differentiated state of the alveolar epithelial cells in the adult lung in that EGFP expression is under the regulation of, and therefore is dependent on, the expression of either the rat podoplanin or the mouse SP-C BAC transgenes. This contrasts with the permanent labeling of TI or TII cell lineages

with Cre-recombinase-mediated activation of reporter gene expression (32, 33). The CBG and Christmas mice should provide useful additions to the current repertoire of transgenic mice in that these mice are useful for identifying virtually all of the TI and TII cells and for isolating both of these cell types. The persistence of detectable EGFP, even after SP-C mRNA expression is essentially nil, permits tracking of TII cells for at least 2 weeks in culture and allows for tracking of cells for a limited time *in vivo* under varying biologic conditions. Finally, in crosses of CBG with

other genetically defined lines, the high percentage of EGFP-labeled TII cells should prove valuable in the study of mouse models of pulmonary development, function, and disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgments: The authors thank Marina Vayner for mouse care, the UCSF Preclinical Therapeutics Core for the use of the IVIS imaging system, and other members of the pulmonary group at UCSF for valuable discussion and support of this project.

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