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Lineage Tracing

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Lineage tracing is the identification of all progeny of a single cell. Although its origins date back to developmental biology of invertebrates in the 19th century, lineage tracing is now an essential tool for studying stem cell properties in adult mammalian tissues. Lineage tracing provides a powerful means of understanding tissue development, homeostasis, and disease, especially when it is combined with experimental manipulation of signals regulating cell-fate decisions. Recently, the combination of inducible recombinases, multicolor reporter constructs, and live-cell imaging has provided unprecedented insights into stem cell biology. Here we discuss the different experimental strategies currently available for lineage tracing, their associated caveats, and new opportunities to integrate lineage tracing with the monitoring of intracellular signaling pathways.

In lineage tracing, a single cell is marked in such a way that the mark is transmitted to the cell's progeny, resulting in a set of labeled clones. Lineage tracing provides information about the number of progeny of the founder cell, their location, and their differentiation status. Used in classical developmental biology, lineage tracing is increasingly applied to stem cell research and in modeling cellular heterogeneity in cancer. In fact, it is an essential tool in stem cell research because it provides information about how the cell behaves in the context of the intact tissue or organism, as opposed to what it is capable of doing following isolation and transplantation or in vitro culture. The field of stem cell research has traditionally relied on flow cytometry to isolate cells to ever-greater purity; the great advantage of lineage tracing is that it can be performed without prior knowledge of what genes or markers are expressed by the cell of interest.

For any lineage tracer, the key features are that it should not change the properties of the marked cell, its progeny, and its neighbors. The label must be passed on to all progeny of the founder cell, should be retained over time, and should never be transferred to unrelated, neighboring cells. In this Primer, we review different techniques for lineage tracing. We highlight the advantages and limitations of each approach and discuss new developments that allow lineage information to be combined with measurements of intracellular signaling pathways.

Direct Observation

Lineage tracing was pioneered in the 19th century by Charles O. Whitman and his colleagues and successors (Conklin, 1905) (Figure 1A). They were inspired by the realization that cells arise from pre-existing cells, rather than through a process of spontaneous generation. They studied early cleavages in invertebrate embryos by light microscopy. Whitman studied leech development, which involves stereotypical, invariant cell divisions. He traced through direct observation the fate of individual cells from the uncleaved egg to the formation of the embryonic germ layers. He found that from the earliest cleavages, the fates of individual cells were developmentally distinct, each cell giving rise to cells that had specific roles in later development.

The nematode *Caenorhabditis elegans* was among the invertebrate embryos studied by 19th century cell biologists, and they made considerable progress in determining lineage relationships from fixed specimens of *C. elegans*. Development in *C. elegans*, as in the leech, is highly determinate, involving cell-fate decisions that are autonomous (i.e., independent from the cell's surroundings). However, the total number of somatic cells in *C. elegans* is several orders of magnitude smaller than in the leech, which makes the analysis more straightforward. A century after the initial studies, the fate of every cell in the *C. elegans* embryo was determined through the use of time-lapse microscopy with Normarksi differential interference contrast optics, which is nondestructive but still provides high resolution (Sulston et al., 1983).

When lineage tracing is performed by direct observation, the system can be manipulated in a number of ways to examine intrinsic and extrinsic factors that regulate cell-fate decisions. For instance, in *C. elegans*, individual cells are ablated by irradiation with a laser microbeam, and cells that fail to form are the normal descendents of the ablated cell. Lineage analysis in *C. elegans* has also been combined with genetic techniques; in particular, mutant worms, which display specific alterations in particular cell lineages, have been studied. Drugs that block cytokinesis (e.g., cytochalasin B), DNA replication (e.g., aphidicolin), or other cell functions (e.g., cell movement) can also be used to perturb the system experimentally.

The advantages of lineage tracing by direct observation include the speed and ease of establishing the technique and the fact that it is noninvasive. A major limitation is that, for an intact organism, continuous observation requires a transparent embryo with a small number of cells. It is also harder to interpret

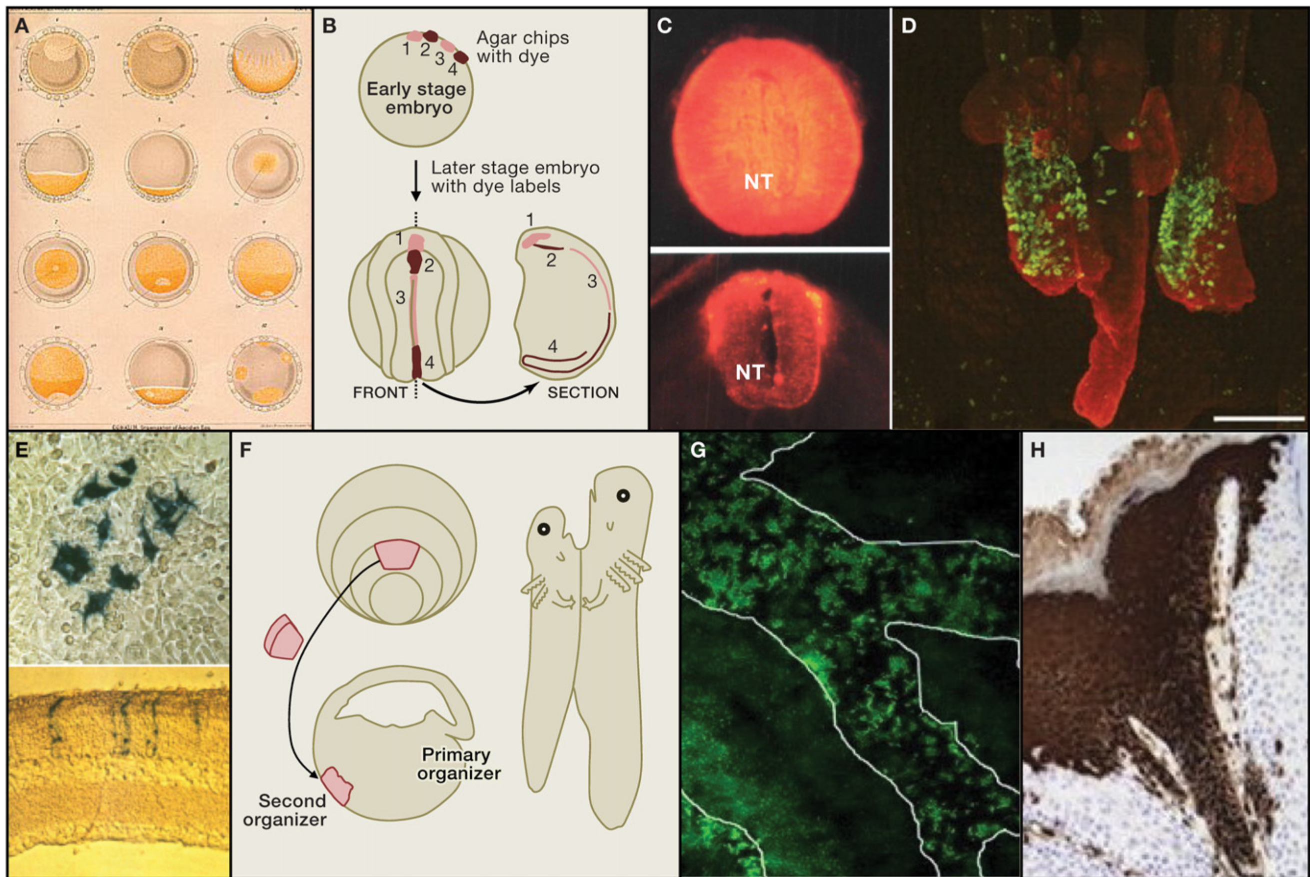

Figure 1. Different Approaches to Lineage Tracing

(A) Direct observation, as pioneered by Whitman and colleagues (exemplified by a plate from Conklin, 1905).

(B) Schematic showing agar chips with vital dyes applied onto the surface of an early stage amphibian embryo (top). These dyes label regions within later stage embryos (bottom) (based on Vogt, 1929; adapted from Gilbert, 2000).

(C) Use of soluble carbocyanine dyes to fate map chick neural crest (reproduced from Serbedzija et al., 1989 with permission from The Company of Biologists Ltd.).

(D) Whole-mount of mouse epidermis showing DNA label-retaining stem cells in the hair follicle bulge (reproduced from Braun et al., 2003). Red: keratin 14; green: BrdU. Scale bar: 100 μm.

(E) *LacZ* retroviral vector introduced into rat retinal cells (upper panel) and subsequently tracked in the reconstituted retina (reproduced from Price et al., 1987 with permission from C. Cepko).

(F) Schematic showing Spemann and Mangold's organizer experiment, which was performed by grafting tissues between amphibian embryos (adapted from Grove, 2008).

(G and H) Adult mouse chimeras from GFP-positive and -negative mice. (G) Whole-mount of lung (reproduced from Giangreco et al., 2009). (H) Histology of skin tumor (reproduced from Arwert et al., 2010). GFP-positive region in (H) is brown.

results when cell-fate decisions are not autonomous. The tissue to be studied must also be accessible, which precludes studies of postimplantation mammalian embryos, unless the cells or embryos are placed in culture, which potentially alters their behavior. Nevertheless, the transparency of zebrafish (*Danio rerio*) embryos has enabled lineage analysis by direct observation to be performed during early vertebrate development (Kimmel et al., 1990).

Although there are limitations to performing lineage tracing by direct observation in whole organisms and tissues, these do not apply to lineage analysis of single cells in culture. Cell biologists have long used time-lapse microscopy to monitor cell division, motility, and death, but the technique can also determine lineage relationships. For example, Temple (1989) isolated precursor cells of the central nervous system from rat embryos (Temple, 1989). By imaging single cells over a number of days, Temple could determine whether the cells divided and formed clones and could assess differentiation status, such as formation of neurons or glia, by cell morphology. In this way, she identified the heterogeneity in cell-fate decisions that is a feature of brain development in mammals but not in the invariant cell divisions of *C. elegans* and leech embryos.

Labeling Cells with Dyes and Radioactive Tracers

When lineage tracing by direct observation is not possible, techniques are required to label the cells of interest. In the early

20th century, "vital dyes," which label cells without killing them, were used to directly mark cells in amphibian embryos and follow the fate of their progeny during gastrulation (Vogt, 1929) (Figure 1B). The technique involved physically marking the cells of interest with a dye impregnated into a small piece of agar. More recently, lipid-soluble carbocyanine dyes (Axelrod, 1979) have been incorporated into the plasma membrane and use for lineage tracing. These include octadecyl (C_{18}) indocarbocyanines and oxacarbocyanine, which are abbreviated as DiI and DiO, respectively. This lineage tracing approach has been use in the neural crest of chicken embryos (Serbedzija et al., 1989) (Figure 1C) and the neural plate of the frog *Xenopus laevis* (Eagleson and Harris, 1990).

In some embryos, such as *Xenopus*, individual cells are large enough to be directly injected with agents, such as fluorescein-conjugated dextran, which cannot diffuse between cells and are an alternative to lipid-soluble fluorescent tracer dyes. Horseradish peroxidase (HRP), which is too large to pass through gap junctions, can be visualized by adding an appropriate substrate for its enzymatic activity. This approach has been used to trace cells at early stages of embryogenesis, particularly in leeches and *Xenopus* (Weisblat et al., 1978). HRP can also be injected into single blastomeres of preimplantation mouse embryos to follow the subsequent allocation of their descendants to the inner cell mass (ICM) and trophectoderm lineages at the 32 cell stage (Bałakier and Pedersen, 1982). Labeling individual cells or a defined group of cells has the major advantage that lineage tracing can be performed in situations that are not amenable to direct visualization by light microscopy. Limitations to this approach include loss of the marker by diffusion or dilution after multiple rounds of cell division.

Some stem cell populations divide infrequently and, if labeled when actively cycling, retain a DNA (e.g., 5-bromo-2′-deoxyuridine) or histone label for many weeks. If these cells subsequently divide, the dilution of label can be used to track the fate of their progeny, such as within the mouse epidermis (Braun et al., 2003; Cotsarelis et al., 1990; Tumbar et al., 2004) (Figure 1D). Analysis of label-retaining cells can also monitor cell turnover in adult human tissues because the level of ^{14}C incorporated into genomic DNA depends on the level in the atmosphere at the time when a cell divides. Nuclear weapon testing during the Cold War led to a marked increase in atmospheric ^{14}C, which then declined exponentially from 1963. Therefore, by knowing when someone was born and how much ^{14}C is present in their cells, it is possible to determine the level of turnover within individual tissues (Spalding et al., 2005; Bergmann et al., 2009).

Introduction of Genetic Markers by Transfection or Viral Transduction

Genetic markers have largely superseded cell marking with HRP or vital dyes. Genetic markers include fluorescent proteins, exemplified by green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (Chalfie et al., 1994) and *Escherichia coli* β-galactosidase (encoded by the *LacZ* gene). The activity of β-galactosidase is visualized by exposure to the substrate X-galactose. Genetic markers can be introduced by direct injection, transfection, or viral infection. Lipofection has been used to transfect *Xenopus* retinal cells with marker genes and map their fate during retina development (Holt et al., 1990), and electroporation has been used to introduce marker genes into chicken and mouse embryos (Itasaki et al., 1999).

Retroviral infection is an efficient method for introducing genes into cells. One of its earliest applications in lineage tracing was to use a *c-myc* retroviral vector to track the fate of hematopoietic stem cells, following transplantation into lethally irradiated recipient mice (Lemischka et al., 1986). Extensive fate mapping of the nervous system has been performed using viral vectors in mammalian and chick embryos (Price et al., 1987; Fekete and Cepko, 1993) (Figure 1E) and to examine the properties of stem cells in adult mouse brain (Doetsch et al., 1999). These studies often combine in vivo and in vitro lineage tracing. One particularly elegant technique uses a transgenic mouse that expresses the avian leukosis virus receptor under the control of a cell-type-specific promoter (Holland and Varmus, 1998). An avian leukosis virus, which encodes alkaline phosphatase (and is replication competent), is then introduced into mice. Only dividing cells that express the receptor become infected and express the lineage marker (Doetsch et al., 1999).

Compared to vital dyes, genetic markers have the advantages that they do not spread to neighboring cells and, if stably expressed, are inherited by the progeny of the marked cell. One potential problem with genetic markers is low efficiency of introducing the gene. However, this issue can be resolved by adding a drug-selectable marker (e.g., puromycin or neomycin) or by using retroviral vectors instead of plasmids. Another caveat is that injecting a vector into tissue can create a wound that can alter cell behavior. In addition, the marker can be toxic to cells, either directly, or indirectly through the exposure to UV light during visualization. Two specific problems with retroviruses are (1) only dividing cells can be labeled, and (2) retroviral vectors can spontaneously silence. Finally, spontaneous cell fusion occurs in a number of tissues (Alvarez-Dolado et al., 2003; Wang et al., 2003). This can result in the transfer of a lineage marker between cells of unrelated lineages (Terada et al., 2002; Ying et al., 2002), leading to the spurious conclusion that transdifferentiation has occurred (Weissman, 2005).

Transplantation of Cells and Tissues

There is a long tradition of mapping cell fate by transplanting tissue from one embryo to a different host, although resolution at the single-cell level is rarely obtained with this approach. In classic experiments, Spemann and Mangold used transplantation to make interspecies chimeras with embryos from three differentially pigmented newt species. They then distinguished the host tissue from the donor tissue based on color (Spemann and Mangold, 1924) (Figure 1F).

Avian interspecies grafts have also yielded important information. Waddington performed interspecies grafting (allotransplantation) with chicken and duck embryos in vitro (Waddington, 1932) to study organ-forming regions during gastrulation, and he could distinguish between host and grafted regions by cell

size. Le Douarin studied migration and differentiation of neural crest cells of the Japanese quail transplanted into chicken embryos. She identified the transplanted quail cells on the basis of structural differences between the interphase nuclei of the two bird species (Le Douarin, 1980).

A different strategy for fate mapping by transplantation is to label cells in the graft with tritiated thymidine and then transplant them into an unlabeled host. Labeled cells from chicken embryos have been transplanted into unlabeled host embryos to study processes such as epiblast formation, early cardiac differentiation, and neural crest cell migration (Rosenquist, 1981).

Transplantation has been used extensively to study stem cell fate in a number of adult mammalian tissues, including blood, muscle, and skin. The classic approach is bone marrow transplantation in sublethally irradiated mice, in which serial reconstitution of blood in successive mice remains the gold standard for assessing stem cell renewal and multilineage differentiation. This can also be applied to the study of single human hematopoietic stem cells, following transplantation into immunocompromised mice (Notta et al., 2011a).

Transplantation has also been used to evaluate stem cells in mouse epidermis: disaggregated adult epidermal cells are combined with neonatal dermal cells in a chamber implanted onto the back of a host mouse and left for several weeks to form hair-bearing skin (Jensen et al., 2010). Transplantation is readily combined with lineage tracing by mixing a small number of marked cells with an excess of unlabeled cells (Jensen et al., 2009).

The advantages of cell transplantation for lineage tracing are 2-fold: there are a number of options for distinguishing between host and donor; and, in the case of interspecies grafts, the label is permanent. Limitations of this approach include the need for surgery, irradiation, or the creation of a wound, which all have the potential to change the properties of the cells under study. There is also the possibility of artifacts due to interspecies differences in cell behavior; indeed interspecies chimeras rarely survive after birth. Furthermore, achieving lineage tracing at single-cell resolution can be a challenge, unless the tissue is blood or can be disaggregated into a single-cell suspension prior to transplantation.

Perhaps the most important disadvantage of using cell transplantation for following cell fate is that single cells may not behave in the context of a graft as they do during normal tissue homeostasis (Watt and Jensen, 2009). This is particularly an issue when performing lineage tracing of epithelial cells, which are normally organized into sheets of cells connected by extensive intercellular junctions but are disaggregated into single-cell suspensions for transplantation. In a recent study, three different populations of epidermal cells showed equal ability to contribute to all epidermal lineages on transplantation, but the populations exhibited more restricted lineage choices in the context of the intact tissue (Watt and Jensen, 2009). Similarly, in the mammary gland, single-cell transplantation experiments have shown the existence of multipotent stem cells. However, lineage tracing in undamaged tissue indicates that the mammary epithelium is normally maintained by stem cell populations whose progeny differentiate along individual lineages (Van Keymeulen et al., 2011).

Genetic Mosaics

As with direct cell observation and tissue grafting, the creation of genetic mosaics is a classic tool for embryonic fate mapping, which continues to have broad applications today. In *Drosophila*, fate mapping was originally based on the experimental generation of gynanders, or flies with tissues that form a mosaic of male and female cells. Subsequently genetic recombination between homologous chromosomes during mitosis in somatic tissue was used to mark individual cells or small groups of cells, as exemplified by cell lineage analysis in the *Drosophila* compound eye.

In the mid-20th century, Tarkowski and Mintz pioneered the analysis of cell fate by generating chimeric mice. They aggregated two eight-cell embryos to form a single embryo that was subsequently implanted into a foster mother and allowed to develop to term (Mintz, 1965). Their studies elucidated the clonal origin of melanocytes and the basis of coat color in adult mice. Different markers can distinguish the contributions of different mouse strains within a chimera. For example, monoclonal antibodies to H-2 cell-surface antigens or lectins can bind cell-surface carbohydrates in one mouse strain but not the other. Studies of chimerism produced some of the earliest cell lineage tracing data in the mouse epidermis and small intestine. These data challenged the prevailing views, which were based on microscopy and label-retaining cells, about how the stem cell compartment of these adult tissues is organized (Schmidt et al., 1987, 1988).

Chimeric mice continue to be used to examine tissue organization. Typically, chimeras are formed between a mouse that expresses GFP in all tissues and one that does not. When combined with whole-mount labeling, the stem cell organization of complex epithelia, such as the lung, can be readily visualized (Giangreco et al., 2009) (Figure 1G). Analysis of chimeric mice has also been used to demonstrate that epidermal tumors are not obligatorily clonal (i.e., derived from a single cell) (Arwert et al., 2010; Winton et al., 1989) (Figure 1H) and to examine how cells bearing oncogenic mutations impact on neighboring cells that are genetically normal (Arwert et al., 2010).

Just as chimeras and genetic mosaics have their origins in the mid 19th century, early cell biologists were intrigued by how cells from different tissues segregate when aggregated with one another in culture (Steinberg, 2007). In a sense, these studies represent chimerism in vitro. Placing different cultured cell populations in contact with one another has been used extensively to examine the molecular basis of intercellular communication. It has also been used for lineage analysis in reconstituted human epidermis. For instance, Lowell et al. (2000) labeled single human epidermal cells with GFP and then examined their clonal growth in combination with an excess of unlabeled cells. This experiment demonstrated that the Notch ligand, Delta-like 1, which is highly expressed on stem cells, stimulates neighboring cells to differentiate and encourages stem cells to adhere to one another (Lowell et al., 2000; Watt et al., 2008).

Naturally occurring genetic mosaics are one of the few tools available for lineage tracing in humans. Early studies relied on extremely rare individuals, such as an XO/XY male with familial

polyposis coli (FAP), a mutation in the adenomatous polyposis coli gene. Using in situ hybridization of biopsy material with Y chromosome probes, Wright and coworkers established that the crypts in the human colon are clonal, that the colon contains multipotential clones, and that early tumors in the intestine are polyclonal (Novelli et al., 1996). A newer strategy exploits the fact that, with increasing age, individual cells within human tissues become deficient in the mitochondrial DNA (mtDNA) encoded enzyme cytochrome *c* oxidase. Enzyme histochemistry to detect cytochrome *c* oxidase can be used as a lineage tracer, and the dynamics of stem cell behavior and clonal succession can be measured (Greaves et al., 2006; McDonald et al., 2008).

Another intriguing approach for lineage tracing in humans is to study revertant phenotypes. Somatic loss of disease-causing mutations, which leads to normalization at the single-cell level, is a rare event, but it can be observed in a number of human skin diseases. One striking example is a skin condition known as ichthyosis with confetti (Choate et al., 2010). Children with the condition are covered in red, flaky skin, but over time, clones of normal epidermis appear, giving adult skin the appearance of being showered with confetti. The disease-causing mutations are frameshift mutations in *keratin 10* (KRT10). The high frequency of somatic reversion in the disease may be due to revertant stem cell clones having a selective growth or survival advantage over mutant cells.

Recently, the clonal architecture of certain human leukemias has been determined. The approach makes use of the fact that a dominant genetic lesion drives initial tumor formation, and a small number of secondary genomic lesions are acquired subsequently (Anderson et al., 2011; Notta et al., 2011b). Therefore, individual clones of leukemic cells can be identified using methods, such as multiplexed fluorescence in situ hybridization, to detect the combination of genetic lesions they contain. These studies have revealed that leukemic cells have a dynamic clonal architecture, with clones and subclones exhibiting competitive regenerative capacity (Anderson et al., 2011; Notta et al., 2011b). Predictions regarding lineage relationships between clones of leukemic cells with different mutations can then be tested experimentally by transplantation in immunocompromised mice (Notta et al., 2011a, 2011b).

Lineage tracing by chimerism has a number of obvious advantages, particularly in its application to human tissues and diseases. In chimeric mice, the sizes of marked regions depend on the number of cells in the blastocysts, which were fused, and thus, the level of resolution is generally confined to groups of cells, rather than individual cells. Nevertheless, single-cell resolution is obtained in some situations, such as somatic reversion and the analysis of leukemic clones. Chimeric mouse tissues with fluorescent reporters are ideal for whole-mount analysis; however, in human tissue, the approach relies on conventional histology or in situ hybridization and is therefore more laborious.

Cell Marking by Genetic Recombination

Genetic recombination has been used for lineage tracing since the early 1990s and is now the preferred approach in most situations. A recombinase enzyme is expressed in a cell- or tissue-specific manner to activate the expression of a conditional reporter gene, and thus permanent genetic labeling of all progeny of the marked cells is achieved. Two site-specific recombination systems adapted from bacteriophage P1 (*Cre-loxP*) and *Saccharomyces cerevisiae* (*FLP-FRT*) have been widely employed, in combination with different conditional reporter genes in a range of organisms (Figure 2).

FLP-FRT recombination has been used mainly to lineage trace cells in *Drosophila* (Figure 2A). The Flippase (FLP) recombinase is expressed under the control of a heat shock-inducible tissue- or cell-specific promoter. Through recombination, the ubiquitously expressed *α-tubulin* promoter on one chromosome is fused with a reporter gene (e.g., *LacZ* or *GFP*) on the homolog at the *flippase recognition target* (*FRT*). Activation of the reporter occurs in only those cells in which the promoter is active. *FLP-FRT* technology was used first to label clones in the *Drosophila* ovary (Harrison and Perrimon, 1993) and then later in a number of other tissues, such as the identification of stem cells in the posterior midgut of adult *Drosophila* (Ohlstein and Spradling, 2006).

Genetic lineage tracing in mice is usually performed using the *Cre-loxP* system (Figure 2B). Cre recombinase is expressed under the control of a tissue- or cell-specific promoter in one mouse line. That line is crossed with a second mouse line in which a reporter is flanked by a *loxP-STOP-loxP* (“floxed” *STOP*) sequence. In animals expressing both constructs, Cre specifically activates the reporter in cells that express the promoter, by excising the *STOP* sequence (Figure 2B). This strategy has now been applied to many tissues, including the epidermis. For example, Fuchs and colleagues placed *Cre* under the control of *Sox9*, a marker specifically expressed in hair follicle bulge stem cells. They then used this construct to trace the origin of bulge stem cells and their progeny during epidermal morphogenesis, showing that *Sox9*-positive cells can give rise to all epidermal lineages (Nowak et al., 2008).

Ubiquitously expressed reporter constructs are usually expressed from the *Rosa26* locus (Figures 2B and 3A). The first reporter was β-galactosidase (Soriano, 1999), which can be visualized when tissue histology is well preserved (Figure 3C). However, β-galactosidase has the limitation that it cannot readily be detected by flow cytometry of live cells. The first fluorescent reporter mouse expressed enhanced GFP (EGFP) (Mao et al., 2001), but many other fluorophores are now available, such as enhanced yellow fluorescent protein (EYFP) and enhanced cyan fluorescent protein (ECFP) (Srinivas et al., 2001).

tdTomato is one of the brightest fluorescent proteins currently available. In the *Rosa26-CAG-tdTomato* reporter mouse (Figure 2B), tdTomato is also targeted to the *Rosa26* locus; however, this locus permits the insertion of an exogenous strong promoter (Muzumdar et al., 2007; Zong et al., 2005), and thus, the ubiquitously expressed *CAG* promoter was added to drive higher expression (Madisen et al., 2010). As a further improvement, the *Rosa26-CAG-tdTomato* reporter construct contains a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to enhance the stability of mRNA. *Rosa26-CAG-tdTomato* mice can be bred

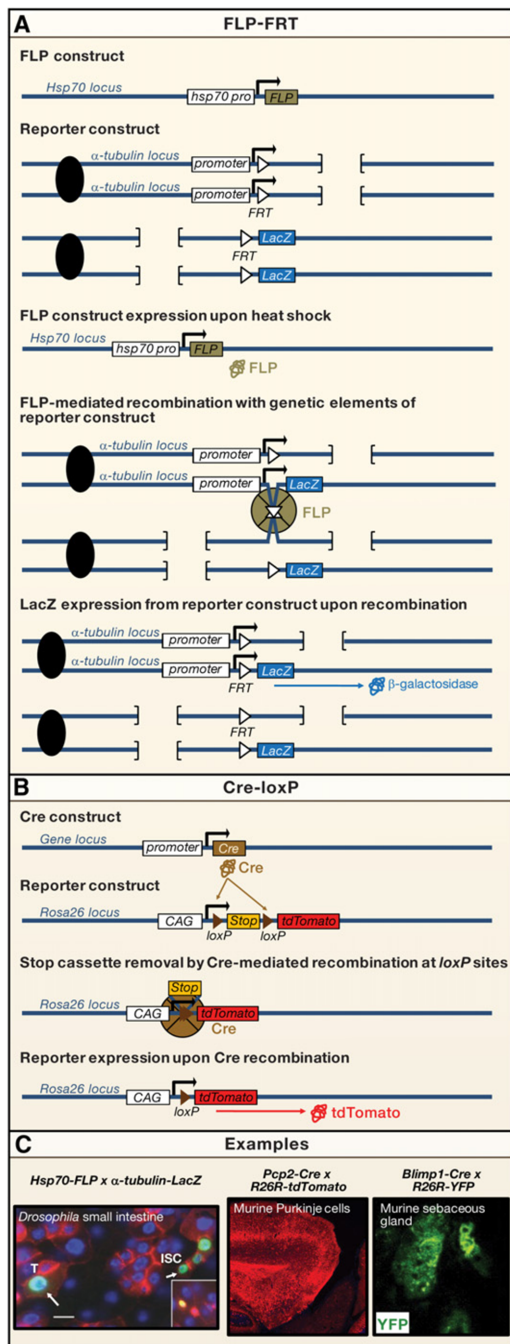

Figure 2. Lineage Tracing through Genetic Recombination

(A) Schematic representation of the genetic elements in the *FLP-FRT* system. Flippase (FLP) recombinase is expressed upon heat shock activation of the gene promoter and fuses the *LacZ* reporter construct with the *α-tubulin* promoter by interchromosomal recombination at FRT sites. As a result, β-galactosidase is expressed and can be visualized by adding X-galactose to the tissue.

(B) Schematic representation of the genetic elements in the *Cre-loxP* system. Cre recombinase is constitutively expressed under the control of a tissue- or (stem) cell-specific promoter. In those cells, Cre can recombine *loxP* sites in the ubiquitously expressed reporter construct to remove the *STOP* cassette. Upon removal of the *STOP*, the reporter (e.g., tdTomato) is expressed in these cells and all their progeny.

(C) An example of FLP recombination is the lineage tracing of intestinal stem cells in *Drosophila* by Ohlstein and Spradling (2006) (left image; image reproduced from *Nature*, © 2006, Macmillan Publishers Ltd.). Examples of Cre recombination are given: tdTomato-labeled Purkinje cells (middle image; Madisen et al., 2010; image reproduced from *Nat. Neurosci.*, © 2010, Macmillan Publishers Ltd.) and EYFP-labeled sebocytes (right image; Horsley et al., 2006).

to homozygosity. Also, the tdTomato fluorophore has strong epifluorescence, which—as another advantage—can be easily visualized. This is useful for either tracking a small number of labeled cells within a tissue or robust labeling for analysis. For sorting labeled populations using flow cytometry, the strong epifluorescence can be disadvantageous because the brightness of tdTomato can bleed into other detection channels, making it difficult or even impossible to sort when cells are colabeled with other markers. Therefore, one has to choose an appropriate reporter mouse line depending on the type of analysis performed.

Temporal and spatial control of Cre activity can also be controlled with inducible recombination, for example, to selectively activate Cre in adult mice via a promoter that is also expressed during embryonic development (Figure 3A). Cre recombinase is typically fused to the human estrogen receptor (ER). In the absence of ligands, such as estrogen 17β-oestradiol, the anti-estrogen tamoxifen, or its active metabolite 4-hydroxy-tamoxifen (4-OHT), the Cre recombinase-ER fusion protein (CreER) is kept in the cytoplasm by heat shock proteins (hsp) (Figures 3A and 3B). Upon application, the ligand diffuses into the cell cytoplasm and binds to the ER. The receptor then changes confirmation, leading to a release from its hsp chaperones. Activated CreER translocates to the nucleus, where Cre can recombine the *loxP* sites (Metzger et al., 1995).

To prevent CreER from being activated by endogenous 17β-oestradiol, two different mutants of CreER have been generated. Parker, Evan and colleagues mutated the mouse ER (*CreER*^{TAM}) (Littlewood et al., 1995), whereas Chambon and colleagues mutated the human ER (*CreER*^{T}) (Feil et al., 1996). Both systems showed insufficient CreER induction at low tamoxifen levels and, thus, required high levels that were sometimes toxic to the cell. This problem was reduced by developing a second generation *CreER*^{T2} construct (Feil et al., 1997). This fusion protein is sensitive to low levels of tamoxifen and displays reduced leakiness because it is not activated by endogenous mouse 17β-oestradiol.

A different system to induce Cre is to fuse it with the progesterone receptor (CrePR), which is activated by applying the anti-progestins Org 31376 or Org 31806 (Tsujita et al., 1999). This construct was improved by developing a mutated version of PR, which is activated by RU486 (mifepristone) (Kyrkanides et al., 2003). Due to reported leakiness of this mutated PR (i.e., CrePR1), Lin and coworkers recently generated a mouse line with a differentially mutated PR fused to Cre recombinase (*GCrePR1e*), and these mice do not exhibit any significant sensitivity to activation by endogenous progesterone (Chen et al., 2010). Still, leakiness is a common problem of inducible Cre systems, whether CreER or CrePR. Therefore,

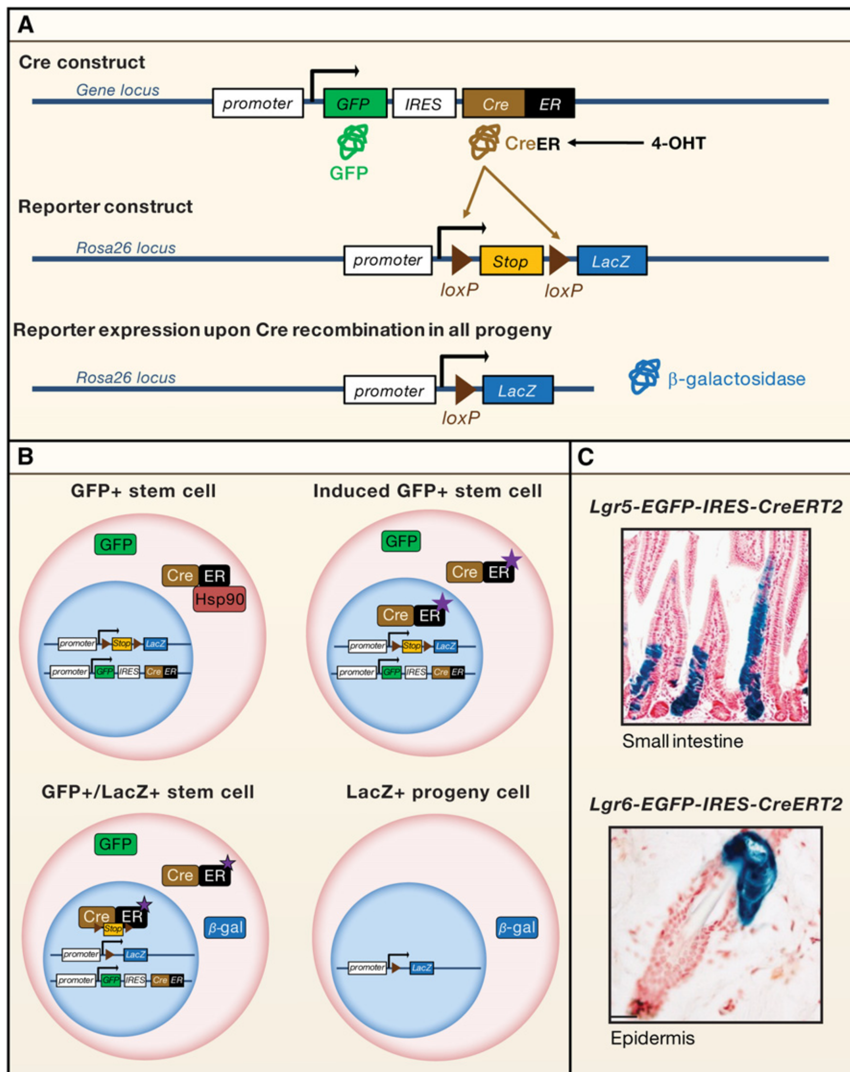

Figure 3. CreER, an Inducible Genetic Recombination System

(A) Schematic representation of the genetic elements. Cre recombinase is fused to a tamoxifen-inducible mutated estrogen receptor (CreER^{T2}). Barker et al. (2007) developed a construct in which CreER^{T2} is expressed in conjunction with a fluorescent reporter (GFP) to visualize the areas of Cre expression. An internal ribosome entry site (IRES) was introduced to allow expression of CreER^{T2} independent of GFP given the length of the overall mRNA transcribed.

(B) Stem cells express GFP and CreER^{T2}, the latter being kept inactive in the cytoplasm by heat shock proteins (Hsp90; upper left panel). Upon binding of tamoxifen or its active metabolite 4-hydroxytamoxifen (4-OHT; asterisk), CreER^{T2} is released from the chaperone and translocates to the nucleus (upper right panel). Cre recombines at *loxP* sites to remove the *STOP* cassette enabling *LacZ* expression (lower left panel). All progeny of those GFP^{+}/LacZ^{+} stem cells express *LacZ* as a genetic mark (lower right panel).

(C) Using this construct, Clevers and colleagues identified stem cells expressing Lgr5 in the small intestine (upper image; Barker et al., 2007, image reproduced with permission from *Nature*, © 2007, Macmillan Publishers Ltd.) and other organs, as well as Lgr6-expressing stem cells in the epidermis (lower image; Snippert et al., 2010a, image reproduced with permission from AAAS.).

it is essential to analyze tissue that has not been exposed to the inducing agent in order to determine the extent of nonspecific activation.

The use of inducible Cre for lineage tracing has provided unprecedented levels of information about the organization of the stem cell compartment in postnatal tissues. Using CrePR1 driven by the *keratin 15* promoter (*K15-CrePR1*), Cotsarelis and colleagues showed that the hair follicle has a population of stem cells that have the capacity to generate all epidermal layers during normal hair follicle cycling (Morris et al., 2004). Recent studies by the Clevers Lab and collaborators led to the identification of *Lgr5*, which is expressed in epithelial stem cells in different tissues, including the intestine (Barker et al., 2007) (Figure 3C), stomach (Barker et al., 2010), and hair follicle (Jaks et al., 2008). Their construct, which is knocked into the *Lgr5* locus, expresses not only CreER^{T2} but also EGFP. This enables the tracking and isolation of stem cells and their progeny by flow cytometry at any time point (Barker et al., 2007; Snippert et al., 2010a). It is important to optimize the dose of the Cre activator (e.g., tamoxifen) for each mouse line expressing inducible Cre. Extremely low doses can be given to label cells at clonal density in order to study origins of particular cell populations. In contrast, high doses are applied to maximize labeling of an entire stem cell population.

When using the *Cre-loxP* approach for lineage tracing, it is important to carefully evaluate where the Cre-driving promoter is expressed. The same promoter can be used for lineage tracing in different tissues, as in the case of *Lgr5* (Barker et al., 2007, 2010; Jaks et al., 2008). However, problems arise when, within an individual tissue, the reporter is expressed by more than one cell population. Thus, within the epidermis, *Blimp1/Prdm1* is a marker of sebocyte progenitors (Horsley et al., 2006), but it is also expressed in terminally differentiated cells of all epidermal compartments, including the sebaceous gland (Lo Celso et al., 2008; Magnúsdóttir et al., 2007). Plus, there is currently no evidence that the sebocyte progenitors give rise to the cells of the interfollicular epidermis. To overcome this type of problem, Kirchhoff and colleagues have developed the "split-Cre" system (Hirrlinger et al., 2009), in which inactive Cre fragments are expressed by two different promoters and the fragments are recombined only in the case of overlapping expression.

An alternative to driving *Cre* expression in a specific subpopulation of cells is to label single cells at random and follow

their fate. Early reporters that used an upstream sequence from the gene of interest, rather than knocking *Cre* into the endogenous locus, tended to suffer from a lack of expression fidelity (i.e., leakiness), with cells activating CreER in the absence of tamoxifen treatment (Vasioukhin et al., 1999). In the case of *K14-CreER*^{TAM} mice (Vasioukhin et al., 1999) crossed with EGFP reporter mice, it was possible to follow the fate of marked clones of cells in untreated epidermis and thereby compare the contribution of different stem cell populations in an unbiased way (Jensen et al., 2009). Conversely, the inefficiency of tamoxifen induction of *K14-CreER*^{TAM} has been used successfully for lineage tracing (Silva-Vargas et al., 2005). *Cre* reporter mice can readily be used to determine the effect on cell lineages of modifying intrinsic or extrinsic signals and to test the effects of disease or damage. For example, tamoxifen can simultaneously induce Cre-mediated recombination and activate Wnt signaling by a truncated β-catenin fused to an ER^{TAM} (*ΔNβ-cateninER*^{TAM}) transgene (Silva-Vargas et al., 2005).

A more sophisticated approach for unbiased clonal lineage tracing has employed a transgenic mouse line, *AhCreER*, in which *CreER* is expressed under the control of the drug-activated *Ah* promoter. Cre is controlled at two levels to minimize leakiness: transcriptionally by treatment with β-napthoflavone, and at the level of protein activity by tamoxifen (Clayton et al., 2007; Kemp et al., 2004). By applying statistical analysis to the data obtained from these experiments, evidence has emerged for stochastic fate decisions and clonal drift within adult tissues such as the epidermis (Clayton et al., 2007).

Lineage Tracing with Multicolor Reporters

Multicolor reporter constructs are being used increasingly for lineage tracing with two or more markers. These constructs make it possible to examine how different cell types contribute to the maintenance and repair of a given tissue (Rinkevich et al., 2011). Mosaic analysis has been performed with double markers (MADM), such as EGFP and red fluorescent protein (RFP) (Figure 4A), to characterize the contribution of certain progenitors to the different layers of the cerebellar cortex (Zong et al., 2005) or to identify the cellular origin of gliomas in mice (Liu et al., 2011). Mosaic analysis with EGFP and LacZ as double markers revealed that epithelial stem cells in *Drosophila* ovarian follicles can migrate across the niche barrier to replace neighboring stem cell pools (Nystul and Spradling, 2007). Luo and colleagues designed a membrane-targeted tdTomato/membrane-targeted EGFP (mT/mG) double-fluorescent reporter mouse in which tdTomato is expressed before a Cre-mediated recombination event whereas EGFP is expressed after the event (Muzumdar et al., 2007). Goldhamer and colleagues combined *Cre-loxP* and *FLP-FRT* recombination to generate a multifunctional *Cre/FLP* dual reporter, which conditionally expresses nuclear β-galactosidase (flanked by *FRT* sites) and EGFP (Yamamoto et al., 2009). Upon Cre recombination, nuclear β-galactosidase is expressed and can be converted into an EGFP reporter by FLP-mediated removal of the *FRT*-flanked LacZ cassette. Germline excision of the *floxed STOP* cassette generates a FLP-dependent EGFP reporter that expresses β-galactosidase before recombination by FLP at *FRT* sites. When Cre and FLP are simultaneously expressed or activated, random recombination enables dual labeling with β-galactosidase and EGFP and therefore tracing of multiple clones (Yamamoto et al., 2009).

The power of lineage tracing with multicolor reporters is illustrated by the demonstration that mouse digit tip regeneration depends on contributions from several different cell types, rather than a single, undifferentiated population of cells (Rinkevich et al., 2011). A widely anticipated reporter system, the "Brainbow" mouse (Livet et al., 2007), enables combinatorial expression of four fluorescent proteins in a stochastic manner. Using incompatible *lox* variants, four fluorescent proteins can be expressed by stochastic recombination using Cre-mediated inversion (Brainbow-2). When three transgenes of a Brainbow construct expressing three "XFPs" (Brainbow-1.0) are introduced into a mouse, independent recombination of those transgene copies can generate ten distinct color combinations (Figure 4B).

In a recent development, the Brainbow-2.1 construct has been used to target the *Rosa26* locus to make a reporter mouse ubiquitously expressing the construct (i.e., the *Rosa26-Confetti*-Mouse) (Snippert et al., 2010b) (Figure 4C). Clevers and coworkers used the reporter mouse to randomly label Lgr5-positive stem cell-derived clones within the intestinal crypt with different fluorophores. Initially multicolored clones were observed, but over time each crypt became labeled with a single color as it became populated by cells derived from a single clone. The authors concluded that homeostasis of murine intestinal crypts is maintained by symmetrically dividing Lgr5-positive stem cells, through neutral drift/clonal succession (Snippert et al., 2010b).

An important aspect of lineage tracing with fluorescent reporter mice is preparing the tissue to ensure that the fluorescent protein can be visualized after tissue processing. Reliable antibodies, such as anti-GFP and anti-RFP/tdTomato/DsRed, that function on fixed, paraffin-embedded or unfixed, frozen sections are available (Jensen et al., 2009; Van Keymeulen et al., 2011). One limitation of multicolor reporter lineage tracing is that costaining the tissue with antibodies to specific markers of differentiation is often not possible.

An alternative to antibody labeling is to visualize the endogenous fluorescence of the reporter (Snippert et al., 2011). This is necessary for tracing multiple fluorescent reporters in thick tissue specimens. It also raises the possibility of imaging clones of cells in living tissue. Such dynamic analysis of cell-fate decisions is facilitated by advances in imaging, such as two- or multiphoton fluorescence microscopes, which enable in-depth scanning and optimal fluorophore separation of the multicolor fluorescence.

Outlook

There are a number of applications of lineage tracing that we anticipate will be developed in the near future. Lineage tracing will be increasingly combined with the manipulation (Jensen et al., 2009; Silva-Vargas et al., 2005; Vooijs et al., 2007) or monitoring of the activation state of specific intracellular signaling pathways. This effort will gather pace as the sensitivity

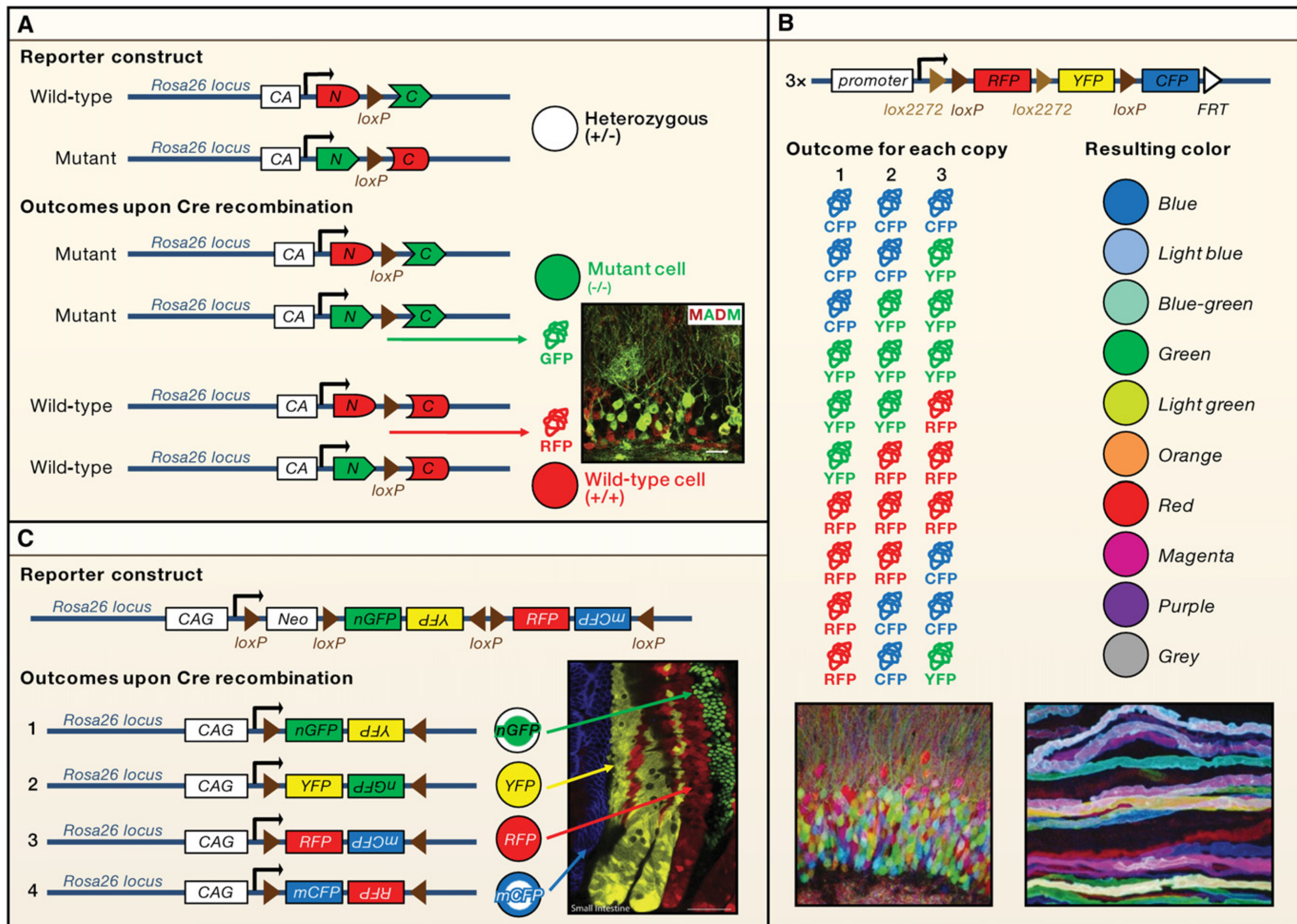

Figure 4. Dual- and Multicolor Reporter Systems

(A) Mosaic analysis with dual markers (MADM). DNA fragments encoding fluorescent proteins (EGFP and tdTomato) are separated by *loxP* sites. Upon Cre-mediated mitotic recombination, the sequence fragments are reconstituted restoring the full-length GFP and tdTomato (Liu et al., 2011; Zong et al., 2005; image reproduced from Liu et al., 2011.).

(B) Brainbow mouse in which ten different colors are generated upon random recombination. Livet et al. (2007) developed a transgene harboring three spectrally distinct fluorescent proteins (Brainbow-1.0). Introducing this trichromatic transgene into mice enables random Cre recombination at *loxP* sites to express several combinations of the three fluorescent proteins. Images show combinatorial expression in dentate gyrus (left) and oculomotor axons (right) (Livet et al., 2007, images reproduced with permission from *Nature* © 2007, Macmillan Publishers Ltd.).

(C) Confetti-Mouse, a stochastic multicolor Cre-reporter. Clevers and colleagues inserted the Brainbow-2.1 construct encoding four fluorescent proteins (Livet et al., 2007) driven by the strong *CAG* promoter into the *Rosa26* locus (Snippert et al., 2010b). Upon Cre recombination, the Neomycin (Neo) cassette is removed and the multicolor construct recombines randomly to result in four possible outcomes with different fluorescent proteins being expressed (image shows clonal expression of the four fluorescent proteins in small intestine; Snippert et al., 2010b).

and fidelity of in vivo reporters improve (DasGupta and Fuchs, 1999; Duncan et al., 2005; Li and Watt, 2005). The combination of fluorescent and bioluminescent reporters in a single cell is one approach by which this can be achieved (Iglesias and Costoya, 2009). Advances in microscopy will allow direct observation of cells that express lineage reporters in adult living mice and provide new information about how stem cells interact with their environment (Lo Celso et al., 2009). The demonstration that light-mediated induction of protein interactions in living cells can be exploited to induce Cre-mediated recombination (Kennedy et al., 2010), offering unprecedented selectivity in the induction of recombination at the single-cell level. Finally, progress has already been made in examining the contributions and lineage relationships of different cell populations to tumors (Youssef et al., 2010). It is safe to assume that these studies will gather further momentum and also that lineage tracing will be applied to other diseases, not only in mice but also in humans.

Concluding Remarks

In Figure 5, we summarize the different strategies that are currently available for lineage tracing and some of the considerations that influence the choice of strategy. It is important to stress that the approaches are not mutually exclusive. For example, Cre can be introduced into mouse tissues by viral infection (Sutherland et al., 2011), and expression of fluorescent

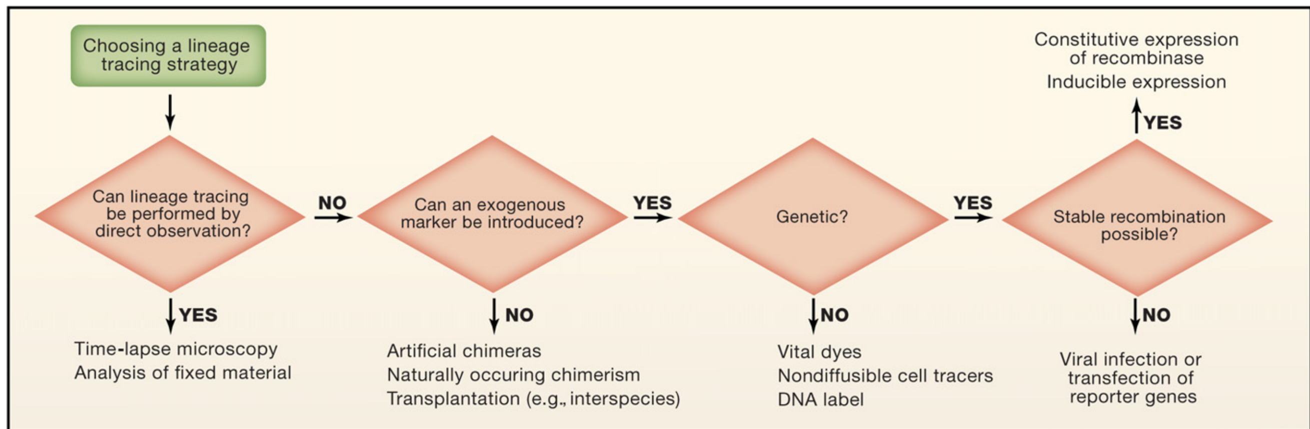

Figure 5. Decision Tree for Choosing an Appropriate Lineage Tracing Strategy
Flow chart summarizing lineage tracing strategies discussed in the text.

reporter genes can be combined with direct observation (Rieger and Schroeder, 2009). Finally, different techniques do not always yield the same results. In particular, a stem cell may exhibit different self-renewal and differentiation properties during normal tissue homeostasis and after transplantation or wound healing (Watt and Jensen, 2009; Kasper et al., 2011). Although we have come a long way since the 19th century, the questions that preoccupy us—How does one cell generate an entire organism? Are cell-fate decisions hard-wired or environmentally regulated? How do cells behave during tissue homeostasis, repair, and disease?—would have been very familiar to Whitman and his colleagues.

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REFERENCES

Alvarez-Dolado, M., Pardal, R., Garcia-Verdugo, J.M., Fike, J.R., Lee, H.O., Pfeffer, K., Lois, C., Morrison, S.J., and Alvarez-Buylla, A. (2003). Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature *425*, 968–973.

Anderson, K., Lutz, C., van Delft, F.W., Bateman, C.M., Guo, Y., Colman, S.M., Kempski, H., Moorman, A.V., Titley, I., Swansbury, J., et al. (2011). Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature *469*, 356–361.

Arwert, E.N., Lal, R., Quist, S., Rosewell, I., van Rooijen, N., and Watt, F.M. (2010). Tumor formation initiated by nondividing epidermal cells via an inflammatory infiltrate. Proc. Natl. Acad. Sci. USA *107*, 19903–19908.

Axelrod, D. (1979). Carbocyanine dye orientation in red cell membrane studied by microscopic fluorescence polarization. Biophys. J. *26*, 557–573.

Bałakier, H., and Pedersen, R.A. (1982). Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. Dev. Biol. *90*, 352–362.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., and Clevers, H. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature *449*, 1003–1007.

Barker, N., Huch, M., Kujala, P., van de Wetering, M., Snippert, H.J., van Es, J.H., Sato, T., Stange, D.E., Begthel, H., van den Born, M., et al. (2010). Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell *6*, 25–36.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., et al. (2009). Evidence for cardiomyocyte renewal in humans. Science *324*, 98–102.

Braun, K.M., Niemann, C., Jensen, U.B., Sundberg, J.P., Silva-Vargas, V., and Watt, F.M. (2003). Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development *130*, 5241–5255.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science *263*, 802–805.

Chen, M.R., Liu, S.W., Wu, T.C., Kao, V.Y., Yu, H.C., Chen, F.H., Hsu, C.H., Chen, C.T., and Lin, K.M. (2010). RU486-inducible recombination in the salivary glands of lactoferrin promoter-driven green fluorescent Cre transgenic mice. Genesis *48*, 585–595.

Choate, K.A., Lu, Y., Zhou, J., Choi, M., Elias, P.M., Farhi, A., Nelson-Williams, C., Crumrine, D., Williams, M.L., Nopper, A.J., et al. (2010). Mitotic recombination in patients with ichthyosis causes reversion of dominant mutations in KRT10. Science *330*, 94–97.

Clayton, E., Doupé, D.P., Klein, A.M., Winton, D.J., Simons, B.D., and Jones, P.H. (2007). A single type of progenitor cell maintains normal epidermis. Nature *446*, 185–189.

Conklin, E.G. (1905). The organization and cell lineage of the ascidian egg. J. Acad. Nat. Sci. Phila. *12*, 1–19.

Cotsarelis, G., Sun, T.T., and Lavker, R.M. (1990). Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell *61*, 1329–1337.

DasGupta, R., and Fuchs, E. (1999). Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development *126*, 4557–4568.

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M., and Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell *97*, 703–716.

Duncan, A.W., Rattis, F.M., DiMascio, L.N., Congdon, K.L., Pazianos, G., Zhao, C., Yoon, K., Cook, J.M., Willert, K., Gaiano, N., and Reya, T. (2005). Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat. Immunol. *6*, 314–322.

Eagleson, G.W., and Harris, W.A. (1990). Mapping of the presumptive brain regions in the neural plate of *Xenopus laevis*. J. Neurobiol. *21*, 427–440.

Feil, R., Brocard, J., Mascrez, B., LeMeur, M., Metzger, D., and Chambon, P. (1996). Ligand-activated site-specific recombination in mice. Proc. Natl. Acad. Sci. USA *93*, 10887–10890.

Feil, R., Wagner, J., Metzger, D., and Chambon, P. (1997). Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. Biochem. Biophys. Res. Commun. *237*, 752–757.

Fekete, D.M., and Cepko, C.L. (1993). Retroviral infection coupled with tissue transplantation limits gene transfer in the chicken embryo. Proc. Natl. Acad. Sci. USA *90*, 2350–2354.

Giangreco, A., Arwert, E.N., Rosewell, I.R., Snyder, J., Watt, F.M., and Stripp, B.R. (2009). Stem cells are dispensable for lung homeostasis but restore airways after injury. Proc. Natl. Acad. Sci. USA *106*, 9286–9291.

Gilbert, S.F. (2000). Developmental Biology, Sixth Edition (Sunderland, MA: Sinauer Associates).

Greaves, L.C., Preston, S.L., Tadrous, P.J., Taylor, R.W., Barron, M.J., Oukrif, D., Leedham, S.J., Deheragoda, M., Sasieni, P., Novelli, M.R., et al. (2006). Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. Proc. Natl. Acad. Sci. USA *103*, 714–719.

Grove, E.A. (2008). Neuroscience. Organizing the source of memory. Science *319*, 288–289.

Harrison, D.A., and Perrimon, N. (1993). Simple and efficient generation of marked clones in Drosophila. Curr. Biol. *3*, 424–433.

Hirrlinger, J., Scheller, A., Hirrlinger, P.G., Kellert, B., Tang, W., Wehr, M.C., Goebbels, S., Reichenbach, A., Sprengel, R., Rossner, M.J., and Kirchhoff, F. (2009). Split-cre complementation indicates coincident activity of different genes in vivo. PLoS ONE *4*, e4286.

Holland, E.C., and Varmus, H.E. (1998). Basic fibroblast growth factor induces cell migration and proliferation after glia-specific gene transfer in mice. Proc. Natl. Acad. Sci. USA *95*, 1218–1223.

Holt, C.E., Garlick, N., and Cornel, E. (1990). Lipofection of cDNAs in the embryonic vertebrate central nervous system. Neuron *4*, 203–214.

Horsley, V., O'Carroll, D., Tooze, R., Ohinata, Y., Saitou, M., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A., and Fuchs, E. (2006). Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. Cell *126*, 597–609.

Iglesias, P., and Costoya, J.A. (2009). A novel BRET-based genetically encoded biosensor for functional imaging of hypoxia. Biosens. Bioelectron. *24*, 3126–3130.

Itasaki, N., Bel-Vialar, S., and Krumlauf, R. (1999). 'Shocking' developments in chick embryology: electroporation and in ovo gene expression. Nat. Cell Biol. *1*, E203–E207.

Jaks, V., Barker, N., Kasper, M., van Es, J.H., Snippert, H.J., Clevers, H., and Toftgård, R. (2008). Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat. Genet. *40*, 1291–1299.

Jensen, K.B., Collins, C.A., Nascimento, E., Tan, D.W., Frye, M., Itami, S., and Watt, F.M. (2009). Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell *4*, 427–439.

Jensen, K.B., Driskell, R.R., and Watt, F.M. (2010). Assaying proliferation and differentiation capacity of stem cells using disaggregated adult mouse epidermis. Nat. Protoc. *5*, 898–911.

Kasper, M., Jaks, V., Are, A., Bergström, A., Schwäger, A., Barker, N., and Toftgård, R. (2011). Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. Proc. Natl. Acad. Sci. USA *108*, 4099–4104.

Kemp, R., Ireland, H., Clayton, E., Houghton, C., Howard, L., and Winton, D.J. (2004). Elimination of background recombination: somatic induction of Cre by combined transcriptional regulation and hormone binding affinity. Nucleic Acids Res. *32*, e92.

Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D., and Tucker, C.L. (2010). Rapid blue-light-mediated induction of protein interactions in living cells. Nat. Methods *7*, 973–975.

Kimmel, C.B., Warga, R.M., and Schilling, T.F. (1990). Origin and organization of the zebrafish fate map. Development *108*, 581–594.

Kyrkanides, S., Miller, J.H., Bowers, W.J., and Federoff, H.J. (2003). Transcriptional and posttranslational regulation of Cre recombinase by RU486 as the basis for an enhanced inducible expression system. Mol. Ther. *8*, 790–795.

Le Douarin, N.M. (1980). The ontogeny of the neural crest in avian embryo chimaeras. Nature *286*, 663–669.

Lemischka, I.R., Raulet, D.H., and Mulligan, R.C. (1986). Developmental potential and dynamic behavior of hematopoietic stem cells. Cell *45*, 917–927.

Li, E.R., and Watt, F.M. (2005). Topo II alpha reporter mice reveal proliferative regions in the epidermis and small intestine. FEBS Lett. *579*, 6479–6485.

Littlewood, T.D., Hancock, D.C., Danielian, P.S., Parker, M.G., and Evan, G.I. (1995). A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. Nucleic Acids Res. *23*, 1686–1690.

Liu, C., Sage, J.C., Miller, M.R., Verhaak, R.G., Hippenmeyer, S., Vogel, H., Foreman, O., Bronson, R.T., Nishiyama, A., Luo, L., and Zong, H. (2011). Mosaic analysis with double markers reveals tumor cell of origin in glioma. Cell *146*, 209–221.

Livet, J., Weissman, T.A., Kang, H., Draft, R.W., Lu, J., Bennis, R.A., Sanes, J.R., and Lichtman, J.W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature *450*, 56–62.

Lo Celso, C., Berta, M.A., Braun, K.M., Frye, M., Lyle, S., Zouboulis, C.C., and Watt, F.M. (2008). Characterization of bipotential epidermal progenitors derived from human sebaceous gland: contrasting roles of c-Myc and beta-catenin. Stem Cells *26*, 1241–1252.

Lo Celso, C., Fleming, H.E., Wu, J.W., Zhao, C.X., Miake-Lye, S., Fujisaki, J., Côté, D., Rowe, D.W., Lin, C.P., and Scadden, D.T. (2009). Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature *457*, 92–96.

Lowell, S., Jones, P., Le Roux, I., Dunne, J., and Watt, F.M. (2000). Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. Curr. Biol. *10*, 491–500.

Madisen, L., Zwingman, T.A., Sunkin, S.M., Oh, S.W., Zariwala, H.A., Gu, H., Ng, L.L., Palmiter, R.D., Hawrylycz, M.J., Jones, A.R., et al. (2010). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. *13*, 133–140.

Magnúsdóttir, E., Kalachikov, S., Mizukoshi, K., Savitsky, D., Ishida-Yamamoto, A., Panteleyev, A.A., and Calame, K. (2007). Epidermal terminal differentiation depends on B lymphocyte-induced maturation protein-1. Proc. Natl. Acad. Sci. USA *104*, 14988–14993.

Mao, X., Fujiwara, Y., Chapdelaine, A., Yang, H., and Orkin, S.H. (2001). Activation of EGFP expression by Cre-mediated excision in a new ROSA26 reporter mouse strain. Blood *97*, 324–326.

McDonald, S.A., Greaves, L.C., Gutierrez-Gonzalez, L., Rodriguez-Justo, M., Deheragoda, M., Leedham, S.J., Taylor, R.W., Lee, C.Y., Preston, S.L., Lovell, M., et al. (2008). Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. Gastroenterology *134*, 500–510.

Metzger, D., Ali, S., Bornert, J.M., and Chambon, P. (1995). Characterization of the amino-terminal transcriptional activation function of the human

estrogen receptor in animal and yeast cells. J. Biol. Chem. *270*, 9535–9542.

Mintz, B. (1965). Genetic mosaicism in adult mice of quadriparental lineage. Science *148*, 1232–1233.

Morris, R.J., Liu, Y., Marles, L., Yang, Z., Trempus, C., Li, S., Lin, J.S., Sawicki, J.A., and Cotsarelis, G. (2004). Capturing and profiling adult hair follicle stem cells. Nat. Biotechnol. *22*, 411–417.

Muzumdar, M.D., Tasic, B., Miyamichi, K., Li, L., and Luo, L. (2007). A global double-fluorescent Cre reporter mouse. Genesis *45*, 593–605.

Notta, F., Doulatov, S., Laurenti, E., Poeppl, A., Jurisica, I., and Dick, J.E. (2011a). Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science *333*, 218–221.

Notta, F., Mullighan, C.G., Wang, J.C., Poeppl, A., Doulatov, S., Phillips, L.A., Ma, J., Minden, M.D., Downing, J.R., and Dick, J.E. (2011b). Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature *469*, 362–367.

Novelli, M.R., Williamson, J.A., Tomlinson, I.P., Elia, G., Hodgson, S.V., Talbot, I.C., Bodmer, W.F., and Wright, N.A. (1996). Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. Science *272*, 1187–1190.

Nowak, J.A., Polak, L., Pasolli, H.A., and Fuchs, E. (2008). Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell *3*, 33–43.

Nystul, T., and Spradling, A. (2007). An epithelial niche in the Drosophila ovary undergoes long-range stem cell replacement. Cell Stem Cell *1*, 277–285.

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature *439*, 470–474.

Price, J., Turner, D., and Cepko, C. (1987). Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc. Natl. Acad. Sci. USA *84*, 156–160.

Rieger, M.A., and Schroeder, T. (2009). Analyzing cell fate control by cytokines through continuous single cell biochemistry. J. Cell. Biochem. *108*, 343–352.

Rinkevich, Y., Lindau, P., Ueno, H., Longaker, M.T., and Weissman, I.L. (2011). Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. Nature *476*, 409–413.

Rosenquist, G.C. (1981). Epiblast origin and early migration of neural crest cells in the chick embryo. Dev. Biol. *87*, 201–211.

Schmidt, G.H., Blount, M.A., and Ponder, B.A. (1987). Immunochemical demonstration of the clonal organization of chimaeric mouse epidermis. Development *100*, 535–541.

Schmidt, G.H., Winton, D.J., and Ponder, B.A. (1988). Development of the pattern of cell renewal in the crypt-villus unit of chimaeric mouse small intestine. Development *103*, 785–790.

Serbedzija, G.N., Bronner-Fraser, M., and Fraser, S.E. (1989). A vital dye analysis of the timing and pathways of avian trunk neural crest cell migration. Development *106*, 809–816.

Silva-Vargas, V., Lo Celso, C., Giangreco, A., Ofstad, T., Prowse, D.M., Braun, K.M., and Watt, F.M. (2005). Beta-catenin and Hedgehog signal strength can specify number and location of hair follicles in adult epidermis without recruitment of bulge stem cells. Dev. Cell *9*, 121–131.

Snippert, H.J., Haegebarth, A., Kasper, M., Jaks, V., van Es, J.H., Barker, N., van de Wetering, M., van den Born, M., Begthel, H., Vries, R.G., et al. (2010a). Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science *327*, 1385–1389.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D., and Clevers, H. (2010b). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell *143*, 134–144.

Snippert, H.J., Schepers, A.G., Delconte, G., Siersema, P.D., and Clevers, H. (2011). Slide preparation for single-cell-resolution imaging of fluorescent proteins in their three-dimensional near-native environment. Nat. Protoc. *6*, 1221–1228.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. *21*, 70–71.

Spalding, K.L., Bhardwaj, R.D., Buchholz, B.A., Druid, H., and Frisén, J. (2005). Retrospective birth dating of cells in humans. Cell *122*, 133–143.

Spemann, H., and Mangold, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Wilhelm Roux'. Arch. Entwicklungsmech. Org. *100*, 599–638.

Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M., and Costantini, F. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev. Biol. *1*, 4.

Steinberg, M.S. (2007). Differential adhesion in morphogenesis: a modern view. Curr. Opin. Genet. Dev. *17*, 281–286.

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

Sutherland, K.D., Proost, N., Brouns, I., Adriaensen, D., Song, J.Y., and Berns, A. (2011). Cell of origin of small cell lung cancer: inactivation of Trp53 and Rb1 in distinct cell types of adult mouse lung. Cancer Cell *19*, 754–764.

Temple, S. (1989). Division and differentiation of isolated CNS blast cells in microculture. Nature *340*, 471–473.

Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D.M., Nakano, Y., Meyer, E.M., Morel, L., Petersen, B.E., and Scott, E.W. (2002). Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature *416*, 542–545.

Tsujita, M., Mori, H., Watanabe, M., Suzuki, M., Miyazaki, J., and Mishina, M. (1999). Cerebellar granule cell-specific and inducible expression of Cre recombinase in the mouse. J. Neurosci. *19*, 10318–10323.

Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W.E., Rendl, M., and Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. Science *303*, 359–363.

Van Keymeulen, A., Rocha, A.S., Ousset, M., Beck, B., Bouvencourt, G., Rock, J., Sharma, N., Dekoninck, S., and Blanpain, C. (2011). Distinct stem cells contribute to mammary gland development and maintenance. Nature *479*, 189–193.

Vasioukhin, V., Degenstein, L., Wise, B., and Fuchs, E. (1999). The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc. Natl. Acad. Sci. USA *96*, 8551–8556.

Vogt, W. (1929). Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Teil Gastrulation und Mesodermbildung bei Urodelen und Anuren. Wilhelm Roux'. Arch. Entwicklungsmech. Org. *120*, 384–706.

Vooijs, M., Ong, C.T., Hadland, B., Huppert, S., Liu, Z., Korving, J., van den Born, M., Stappenbeck, T., Wu, Y., Clevers, H., and Kopan, R. (2007). Mapping the consequence of Notch1 proteolysis in vivo with NIP-CRE. Development *134*, 535–544.

Waddington, C.H. (1932). Experiments on the development of chick and duck embryos cultivated *in vitro*. Philos. Trans. R. Soc. Lond., B *221*, 179–230.

Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., Olson, S., and Grompe, M. (2003). Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature *422*, 897–901.

Watt, F.M., and Jensen, K.B. (2009). Epidermal stem cell diversity and quiescence. EMBO Mol Med *1*, 260–267.

Watt, F.M., Estrach, S., and Ambler, C.A. (2008). Epidermal Notch signalling: differentiation, cancer and adhesion. Curr. Opin. Cell Biol. *20*, 171–179.

Weisblat, D.A., Sawyer, R.T., and Stent, G.S. (1978). Cell lineage analysis by intracellular injection of a tracer enzyme. Science *202*, 1295–1298.

Weissman, I.L. (2005). Normal and neoplastic stem cells. Novartis Found. Symp. *265*, 35–50.

Winton, D.J., Blount, M.A., and Ponder, B.A. (1989). Polyclonal origin of mouse skin papillomas. Br. J. Cancer *60*, 59–63.

Yamamoto, M., Shook, N.A., Kanisicak, O., Yamamoto, S., Wosczyna, M.N., Camp, J.R., and Goldhamer, D.J. (2009). A multifunctional reporter mouse line for Cre- and FLP-dependent lineage analysis. Genesis *47*, 107–114.

Ying, Q.L., Nichols, J., Evans, E.P., and Smith, A.G. (2002). Changing potency by spontaneous fusion. Nature *416*, 545–548.

Youssef, K.K., Van Keymeulen, A., Lapouge, G., Beck, B., Michaux, C., Achouri, Y., Sotiropoulou, P.A., and Blanpain, C. (2010). Identification of the cell lineage at the origin of basal cell carcinoma. Nat. Cell Biol. *12*, 299–305.

Zong, H., Espinosa, J.S., Su, H.H., Muzumdar, M.D., and Luo, L. (2005). Mosaic analysis with double markers in mice. Cell *121*, 479–492.