

Cre-driver lines used for genetic fate mapping of neural crest cells in the mouse: An overview

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Summary

The neural crest is one of the embryonic structures with the broadest developmental potential in vertebrates. Morphologically, neural crest cells emerge during neurulation in the dorsal folds of the neural tube before undergoing an epithelial-to-mesenchymal transition (EMT), delaminating from the neural tube, and migrating to multiple sites in the growing embryo. Neural crest cells generate cell types as diverse as peripheral neurons and glia, melanocytes, and so-called mesectodermal derivatives that include craniofacial bone and cartilage and smooth muscle cells in cardiovascular structures. In mice, the fate of neural crest cells has been determined mainly by means of transgenesis and genome editing technologies. The most frequently used method relies on the *Cre-loxP* system, in which expression of Cre-recombinase in neural crest cells or their derivatives genetically enables the expression of a Cre-reporter allele, thus permanently marking neural crest-derived cells. Here, we provide an overview of the Cre-driver lines used in the field and discuss to what extent these lines allow precise neural crest stage and lineage-specific fate mapping.

KEYWORDS

Cre/LoxP system, lineage tracing, neural crest

1 | A BRIEF SYNOPSIS OF METHODS USED FOR NEURAL CREST CELL FATE MAPPING

The astonishing variety of neural crest derivatives has long been recognized, mainly through the pioneering work by Le Douarin and co-workers who used interspecies transplantation in avian embryos to determine normal fates as well as the developmental potential of neural crest cell populations from different axial levels of the embryo (Bronner & Simões-Costa, 2016; Le Douarin & Dupin, 2003). Together with assays involving dye labeling and retroviral infection of neural crest cells, this approach led to the establishment of comprehensive fate maps and revealed that certain neural crest derivatives (such as peripheral glia, sensory neurons, and melanocytes) are produced from all axial levels, whereas other neural crest cell lineages (such as cartilage, bone, smooth muscle, connective tissue, endocrine cells as well as, for instance, neurons and glia from the enteric and parasympathetic nervous system) originate from discrete levels along the neuraxis.

Given the limited accessibility of mammalian embryos, lineage-tracing experiments as performed in avian embryos have not been widely used for fate mapping of mammalian neural crest cells. Rather, various genetic tools have been established that allow noninvasive and long-term tracing of neural crest cells in mice *in vivo* (Zurkirchen & Sommer, 2017). By far the most frequently used approach in the field is *Cre-loxP*-based conditional genetic recombination that, when combined with a Cre-reporter line, results in inheritable and irreversible expression of a marker gene in Cre-recombinase-expressing cells and in all of their progeny (Woodworth, Girsakis, & Walsh, 2017). Furthermore, inducible forms of Cre-recombinase have been applied to perform stage-dependent fate mapping of neural crest cells and their derivatives or fate mapping at low recombination density for *in vivo* single cell tracing (Baggiolini et al., 2015; Kaucka et al., 2016). Apart from confirming in mice many of the findings obtained by fate mapping of avian neural crest cells, genetic lineage tracing of murine neural crest cells led, for instance, to the identification of minor neural crest-derived cell populations present in tissues of nonneural crest origin, to the

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TABLE 1 List of publicly available neural crest drivers

Premigratory neural crest drivers		
Short name	Official Name	Original study
<i>P3Pro-Cre</i>	Tg(Pax3-cre)1Joe	Li et al. (2000)
<i>Wnt1-Cre</i>	H2afv ^{Tg(Wnt1-cre)11Rth}	Danielian et al. (1998)
<i>Wnt1-Cre2</i>	E2f1 ^{Tg(Wnt1-cre)2Sor}	Lewis et al. (2013)
<i>Wnt1-CreER</i>	Tg(Wnt1-cre/ERT)1Alj	Zervas et al. (2004)
<i>Wnt1-Flpe</i>	Tg(Wnt1-FLP1)1Dym	Dymecki et al. (1998)
<i>Wnt1-FlpeER^{T2}</i>	Tg(Wnt1-flpe/ERT2)9455Dym	Hunter et al. (2005)
Migratory neural crest drivers		
Short name	Official name	Original study
<i>AP2α-IRESCre</i>	<i>Tfap2a</i> ^{tm1(cre)Moon}	Macatee et al. (2003)
<i>Ht-PA-Cre</i>	Tg(PLAT-cre)116Sdu	Pietri et al. (2003)
<i>Mef2c-F10N-Cre</i>	Tg(Mef2c-cre)5Patr	Aoto et al. (2015)
<i>P0-Cre</i>	Tg(Mpz-cre)94Imeg	Yamauchi et al. (1999)
<i>Plp-CreER^T</i>	Tg(Plp1-cre/ERT)3Pop/J	Doerflinger et al. (2003)
<i>Plp-CreER^{T2}</i>	Tg(Plp1-cre/ERT2)1Ueli	Leone et al. (2003)
<i>Sox10-Cre</i>	Tg(Sox10-cre)1Wdr	Matsuoka et al. (2005)
<i>Sox10ER^{T2}CreER^{T2}</i> (SECE)	Tg(Sox10-ERT2/cre/ERT2)17Sor	He and Soriano (2015)
<i>Sox10-iCreER^{T2}</i>	Tg(Sox10-icre/ERT2)1Ldim	Simon et al. (2012)
<i>Sox10-iCreER^{T2}</i>	Tg(Sox10-icre/ERT2)26Vpa	Laranjeira et al. (2011)
<i>Sox10-iCreER^{T2}</i>	Tg(Sox10-icre/ERT2)388Wdr	McKenzie et al. (2014)
<i>TEC1</i>	Tg(Tyr-cre)1Gfk	Tonks et al. (2003)

establishment of novel lineage trees (revealing, in particular, the broad developmental potential of peripheral glial cells), and to the demonstration of in vivo multipotency of single premigratory and migratory neural crest cells. While these studies have recently been covered elsewhere (Petersen & Adameyko, 2017; Zurkirchen & Sommer, 2017), in the present review we aim to focus on the tool set used in the field, summarizing the findings made with and discussing specific properties of various Cre lines that have been utilized to trace neural crest cells.

2 | TRACING OF PREMIGRATORY AND MIGRATORY NEURAL CREST CELLS USING NONINDUCIBLE CRE-DRIVER LINES

A well-established Cre-driver line for neural crest lineage tracing is *Wnt1-Cre* (Danielian, Muccino, Rowitch, Michael, & McMahon, 1998) (Table 1). This transgenic mouse line expresses Cre initially in the mid-brain and, after closure of the neural tube, in the midlines of the mid-brain and the caudal diencephalon, in the midbrain–hindbrain junction, and in the dorsal spinal cord, where it recombines premigratory neural crest cells. By crossing *Wnt1-Cre* mice with the *ROSA26* (*R26R*) Cre-reporter line (that drives β -galactosidase expression upon Cre-mediated recombination) (Soriano, 1999), it was shown that *Wnt1-Cre* is a highly efficient Cre-driver line, resulting in recombination of approximately 96% of all migratory neural crest cells (Hari et al., 2012).

Because *Wnt1* is not expressed in migratory neural crest cells and *Wnt* activity rapidly decreases in neural crest cells after their delamination from the neural tube (Kléber et al., 2005; Rabadán et al., 2016; Zervas, Millet, Ahn, & Joyner, 2004), it can be assumed that most neural crest cells are very efficiently targeted by *Wnt1-Cre* before or at the time of their delamination. Intriguingly, however, despite the early activity of *Wnt1-Cre* in the dorsal neural tube, recombination apparently occurs too late to allow investigation of mechanisms regulating epithelial-to-mesenchymal transition (EMT) or delamination of neural crest cells. Indeed, *Wnt1-Cre*-mediated ablation of signaling pathways shown in other animal models to be crucial for neural crest EMT, such as canonical *Wnt* signaling and signaling by TGF β superfamily factors, did not affect early stages of neural crest development (Brault et al., 2001; Büchmann-Møller et al., 2009; Hari et al., 2002; Jia et al., 2007). In contrast, the differentiation potential of neural crest cells along all axial levels could readily be monitored using *Wnt1-Cre*, both during development and at postnatal stages (Chai et al., 2000; Jiang, Rowitch, Soriano, McMahon, & Sucov, 2000; Zurkirchen & Sommer, 2017). A potential caveat of this line is, however, that, at least in the midbrain, *Wnt1* is ectopically expressed from the *Wnt1-Cre* transgene, which could lead to ectopic activation of canonical *Wnt* signaling (Lewis, Vasudevan, O'Neill, Soriano, & Bush, 2013). Although it is not known whether such ectopic *Wnt1* expression also affects the neural crest, the use of a new driver line termed *Wnt1-Cre2* should be considered (Lewis et al., 2013). In

fact, in studies addressing the role of fibronectin in cardiac neural crest development, considerable phenotypic variances have been reported upon *Wnt1-Cre* vs. *Wnt1-Cre2*-mediated recombination, although this could have been due to differences between the two transgenic lines other than aberrant *Wnt1* expression (Wang & Astrof, 2016).

Apart from the *Cre/loxP* system, another site-specific recombination system has also been established to trace the fate of neural crest cells. To this end, two transgenic mouse lines (termed *Wnt1-Flpe* mice) were independently generated that express Flp recombinase from the *Wnt1* promoter (Dymecki & Tomasiewicz, 1998; Hatzistergos et al., 2015). Although the recombination efficiency and the extent of neural crest lineages traceable by these lines have not been described in detail, these lines were instrumental to perform intersectional lineage tracing of cells that concurrently express two distinct promoters. When combined with either the *RC::FrePe* (Engleka et al., 2012) or *RC::Fela* (Jensen et al., 2008) dual reporter alleles (which report dual Flp and Cre recombination), a fraction of *cKit-CreER^{T2}*-traced cardiac progenitors was shown to derive from the cardiac neural crest (traced by *Wnt1-Flpe*) (Hatzistergos et al., 2015). Likewise, intersectional fate-mapping with the *RC::FrePe* allele was used to demonstrate that *Isl1* is not an exclusive marker for second heart field cardiac progenitors, as previously suggested, but also marks a subpopulation of cardiac neural crest cells (Engleka et al., 2012).

Another mouse line expressing Cre in the dorsal neural tube and premigratory neural crest is *P3Pro-Cre*, in which Cre expression is driven from a *Pax3* promoter fragment (Li, Chen, & Epstein, 2000). Although *Pax3* is expressed in the neural plate border before bona fide neural crest specification (Bronner & Simões-Costa, 2016), Cre-mediated conditional inactivation of pathways controlling EMT/delamination did not affect neural crest cell production and early migration in *P3Pro-Cre* embryos (Buchmann-Moller and Sommer, unpublished). Thus, we are not aware of a Cre-driver line suitable for the study of early events in neural crest development, including neural crest specification, EMT, and delamination. Fate mapping experiments with *P3Pro-Cre* have demonstrated efficient labeling of postmigratory neural crest derivatives, such as the enteric nervous system, the mesenchyme in pharyngeal arches, and cardiovascular structures. In contrast to the *Wnt1-Cre* line, however, *P3Pro-Cre*-mediated recombination appears to be less specific for neural crest lineage tracing as it also marks noncrest neuroepithelial cells and several mesodermal tissues, including cartilaginous portions of the ribs and a large part of the skeletal musculature (Jarad & Miner, 2009; Li et al., 2000; Liu et al., 2006; Lang et al. 2000).

Several Cre-driver lines have been generated that, unlike the *Wnt1-Cre* or *P3Pro-Cre* lines, express Cre-recombinase in neural crest cells not before they undergo an EMT in the dorsal neural tube, but only as the cells begin to migrate. For instance, transgenic *Ht-PA-Cre* mice express Cre under the control of a human tissue plasminogen activator (*Ht-PA*) promoter fragment specifically in migratory neural crest cells (Pietri, Eder, Blanche, Thiery, & Dufour, 2003). A detailed comparison with *Wnt1-Cre/R26R* mice revealed very efficient labeling of neural crest derivatives by *Ht-PA-Cre*, including neuronal, glial, melanocytic, and mesenchymal cell populations during development and in adult structures (Pietri et al., 2003; Wong et al., 2006). *Ht-PA-Cre/R26R* mice

were also reported to label a fraction of nonneural epithelial cells lateral to cranial neural folds that were suggested to contribute to mesecto-dermal structures in the head (Breau, Pietri, Stemmler, Thiery, & Weston, 2008).

Additional Cre-driver lines mostly used to study cranial and cardiac neural crest development are the transgenic line *Mef2c-F10N-Cre* (Aoto et al., 2015) and the *AP2 α -IRESCre* line generated by knock-in of an IRESCre cassette into the 3' untranslated region of the *AP2 α* transcription factor locus (Macatee et al., 2003). A detailed analysis of *Mef2c-F10N-Cre/R26R* embryos at different stages confirmed the neural crest origin of various neural and nonneural structures and emphasized the neural crest origin of olfactory ensheathing glial cells, cells in the meninges surrounding the forebrain, and cells of the choroid plexus. Apart from marking migratory neural crest cells only after delamination from the neural tube, *Mef2c-F10N-Cre/R26R* presented a neural crest fate map highly similar to the one obtained with the *Wnt1-Cre* line, with the exception of some differences apparent in the calvarial bones (Aoto et al., 2015).

Tyrosinase (*Tyr*) is a key enzyme of the melanin biosynthetic pathway and, accordingly, a transgenic mouse line, in which *Tyr* enhancer and promoter elements drive Cre expression, was found to mark the melanocytic lineage, but not other neural crest derivatives (Delmas, Martinozzi, Bourgeois, Holzenberger, & Larue, 2003). In contrast, an independently produced *Tyr-Cre* line termed *TEC1* also marks neural crest lineages other than melanoblasts, such as craniofacial structures, dorsal root ganglia (DRG), and sympathetic cephalic ganglia, albeit at a seemingly low recombination efficiency and at a relatively late stage in neural crest development (embryonic day (E) 10.5 onwards) (Tonks et al., 2003). Whether this reflects activity of tyrosinase transcriptional elements in a subset of undifferentiated migratory neural crest cells or aberrant transgene expression remains to be determined.

Although *P0* was originally identified as a marker of the peripheral glial lineage, the expression of *P0-Cre* in transgenic mice was not lineage restricted, but detected already in migratory neural crest cells (Yamauchi et al., 1999). Consequently, *P0-Cre/R26R* mice displayed β -galactosidase expression in multiple neural and nonneural tissues originating from the neural crest. However, at least in some structures such as the DRG, the recombination efficiency appeared to be considerably lower than the one achieved by *Wnt1-Cre*-mediated recombination. Furthermore, cranial neural crest cell populations are differentially marked in *Wnt1-Cre* vs. *P0-Cre* mice: *Wnt1-Cre* preferentially recombines midbrain as opposed to hindbrain neural crest cells, while hindbrain neural crest cells are efficiently targeted by *P0-Cre* (Chen et al., 2017). Intriguingly, fate-mapping experiments by means of the *P0-Cre* line also allowed the identification of neural crest-derived cells in structures previously not known to harbor such cells. Specifically, Nagoshi and colleagues demonstrated that neural crest cells give rise to vascular endothelial and smooth muscle cells present in the adult bone marrow, which was later confirmed in *Wnt1-Cre* mice (Nagoshi et al., 2008; Wislet-Gendebien et al., 2012). Likewise, between 5 and 15% of all hormone-producing cells in the anterior lobe of the pituitary turned out to originate from the neural crest based on *P0-Cre*- lineage tracing (Ueharu et al., 2017).

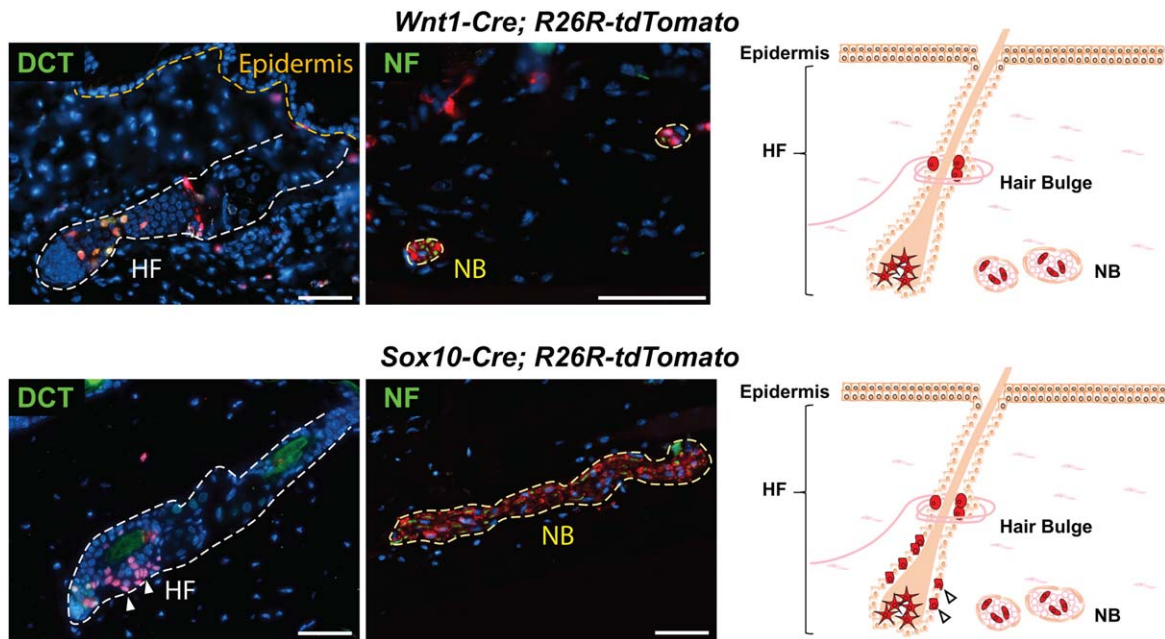


FIGURE 1 Adult skin structures traced by *Wnt1-Cre* and *Sox10-Cre*-driven recombination. Immunolabeling of adult skin from mice, in which recombination of the Cre-reporter allele *R26R-tdTomato* was driven during neural crest development by *Wnt1-Cre* (Danielian et al., 1998) and *Sox10-Cre* (Matsuoka et al., 2005), respectively. Genetically recombined cells are labeled with tdTomato (red) in combination with selected lineage markers (green): DCT (melanocytes in hair follicles (HF)); NF (axons of nerves in nerve bundles (NB)). Aberrant tracing of some keratinocytes (white arrows) can be observed with *Sox10-Cre*. Hoechst nuclei counterstaining, scale bars 50 μ m. The schematics summarize the neural crest lineage-specific and unspecific recombination events observed with these Cre-driver lines. Star-like cells: melanocytes; cells in hair bulges: glial cells and melanocyte stem cells; cells in NBs: Schwann cells

Yet another transgenic mouse line expressing Cre in neural crest cells only once they have engaged in migration is *Sox10-Cre* (Hari et al., 2012; Matsuoka et al., 2005). Fate mapping in *Sox10-Cre/R26R* embryos, combined with immunostaining for Cre protein, demonstrated targeting of approximately 78% of all *Sox10*-positive neural crest cells after their emigration from the neural tube (Hari et al., 2012). Thanks to this high recombination efficiency it was possible to address stage-specific functions of canonical Wnt signaling upon constitutive activation in premigratory neural crest (using *Wnt1-Cre*) vs. migratory neural crest cells (using *Sox10-Cre*). Furthermore, the *Sox10-Cre* line was used together with the *Wnt1-Cre* line to show an unexpected contribution of neural crest cells in the murine neck region to muscle connective tissue, cartilage and bone, including endochondral bones that were till then believed to exclusively originate from the mesoderm (Matsuoka et al., 2005). However, unlike *Wnt1-Cre*, *Sox10-Cre* activity leads to recombination of some adult structures that are not of neural crest origin, such as subpopulations of epithelial cells present in hair follicles (Figure 1).

3 | NEURAL CREST LINEAGE TRACING USING INDUCIBLE CRE-DRIVER LINES

The above-described Cre lines allow *in vivo* fate mapping or functional analysis of a gene of interest only at the first time the Cre-driving promoter is active. To temporally control Cre activity, inducible forms of Cre have been developed, for instance by fusion with a mutant ligand-

binding domain of the human estrogen receptor (CreER) that bind to the synthetic estrogen receptor ligand 4-hydroxytamoxifen (4-OHT) or tamoxifen (TM), but not to endogenous estradiol (Feil et al., 1996). Subsequently, a refined version with a higher TM sensitivity was constructed (termed CreER^{T2}) (Feil, Wagner, Metzger, & Chambon, 1997). Although some mouse lines expressing inducible forms of Cre display leakiness, CreER- and CreER^{T2}-expressing lines have become very valuable tools to determine stage-specific roles of genes of interest, to carry out lineage tracing experiments at different stages of neural crest development, or to perform clonal analyzes of neural crest cells *in vivo* by choosing conditions enabling low recombination frequencies (Zurkirchen & Sommer, 2017).

Because *Wnt1* is only expressed in premigratory neural crest, but not once neural crest cells have emigrated from the neural tube, *Wnt1-CreER* and *Wnt1-FlpeER^{T2}* mice (generated to study lineage relationships of cells at the mid/hindbrain boundary (Hunter, Awatramani, Farley, & Dymecki, 2005; Zervas et al., 2004)) (Table 1) are not suitable for neural crest lineage tracing at different time points. However, the *Wnt1-CreER* line has been successfully used in conjunction with the multicolor Cre-reporter allele *R26R-Confetti* (Snippert et al., 2010) to show that premigratory *Wnt1*-expressing neural crest cells are multipotent *in vivo* (Baggiolini et al., 2015). In this study, low dose TM treatment of pregnant mice led to low density recombination of the multicolor Cre-reporter in neural crest cells homozygous for *R26R-Confetti*, resulting in clones of cells expressing either nuclear green, cytoplasmic yellow, cytoplasmic red, membrane-bound blue, or rare combinations thereof. Intriguingly, around 20% of all the clones derived

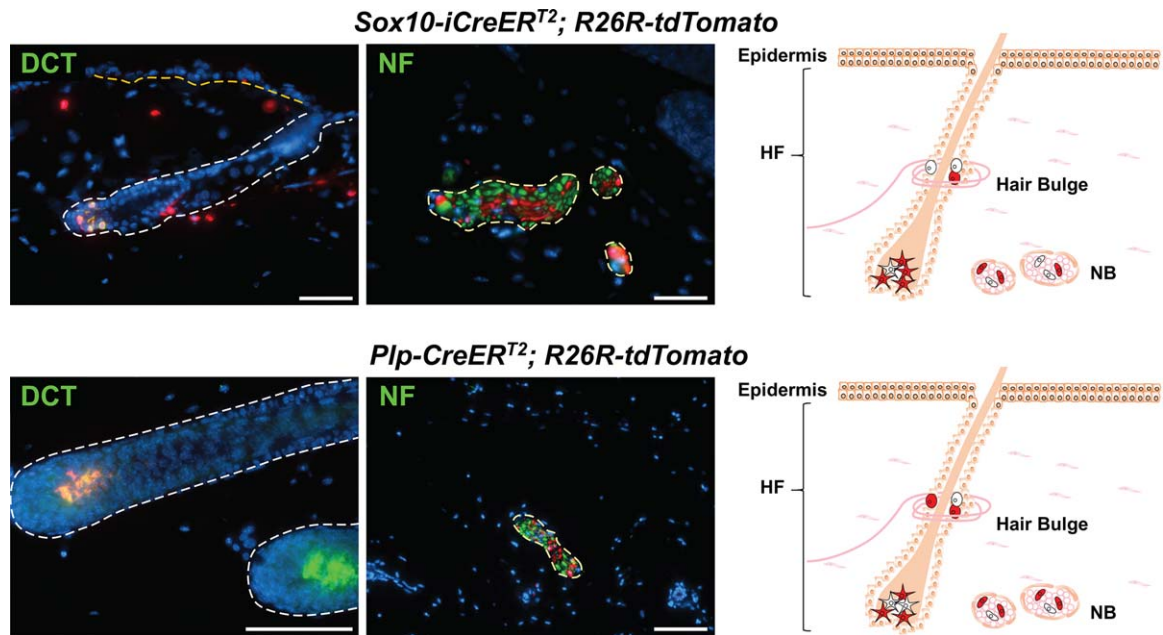


FIGURE 2 *Sox10-iCreER^{T2}* and *Plp-CreER^{T2}*-mediated tracing of adult neural crest-derived cells in the skin. Immunolabeling of adult skin of tamoxifen-free animals carrying inducible *Sox10-iCreER^{T2}* (Simon et al., 2012) and *Plp-CreER^{T2}* (Leone et al., 2003), respectively, reveals leakiness of these driver lines in combination with the Cre-reporter allele *R26R-tdTomato* (red) in melanocyte stem cells and glial cells present in or around the hair bulge, melanocytes (DCT (green); star-like cells in the schematics), and Schwann cells in nerve bundles (NB). NF marks axons of nerves. Hoechst nuclei counterstaining, scale bars 50 μ m. Note that tamoxifen treatment of adult animals results in significantly enhanced *CreER^{T2}* activity in peripheral glia and melanocytes in both *Sox10-iCreER^{T2}* and *Plp-CreER^{T2}* lines (Parfejevs et al., 2018)

from single *Wnt1-CreER*-traced neural crest cells not only contributed to multiple neural crest cell lineages, but also contained daughter cells in the dorsal neural tube. These data are consistent with the idea that at least some premigratory neural crest cells self-renew *in vivo*.

The assumption that multipotent neural crest cells can self-renew during a given time window was further supported by clonal analysis of migratory neural crest cells, traced shortly after emigration by means of a *Sox10-iCreER^{T2}* driver line (Baggiolini et al., 2015; Simon, Lickert, Götz, & Dimou, 2012). By using *R26R-Confetti* as Cre-reporter in *Sox10-iCreER^{T2}* mice treated with low TM doses, the vast majority of migratory neural crest cells were shown to maintain multipotency, and the *Sox10-iCreER^{T2}*-expressing cells did not display any higher degree of fate restriction as compared to their premigratory counterparts (Baggiolini et al., 2015). The only structure labeled by *Wnt1-CreER*- but not *Sox10-iCreER^{T2}*-lineage tracing was the dorsal neural tube, confirming that *Sox10-iCreER^{T2}* is not expressed in the premigratory neural crest, but only once the cells start to migrate. *R26R-Confetti*-based clonal analysis of neural crest cells has also been performed with another, independently produced *Sox10-iCreER^{T2}* line (Kaucka et al., 2016; Laranjeira et al., 2011). In this study, composition and behavior of ectomesenchymal cranial neural crest-derived clones were monitored during early craniofacial development, revealing that cranial neural crest cell-derived clones frequently comprise odontogenic, chondrogenic, osteogenic, and adipogenic cells.

Sox10 is not only expressed in migratory neural crest cells, but its expression is maintained throughout development and postnatally in the glial and melanocyte lineage, including in adult tissue (Bremer et al.,

2011; Kuhlbrodt, Herbarth, Sock, Hermans-Borgmeyer, & Wegner, 1998; Shakhova et al., 2012). Moreover, it marks cells with neural crest stem cell (NCSC) features that have been isolated from various adult neural crest-derived structures (Shakhova & Sommer, 2010). Importantly, reporter expression in *Sox10-iCreER^{T2}* lines nicely matches the known *Sox10* expression pattern during development and in the adult (Laranjeira et al., 2011; Simon et al., 2012). Thus, these *Sox10-CreER^{T2}* lines (Laranjeira et al., 2011; Simon et al., 2012) are ideally suited to monitor whether neural crest-derived cells give rise to distinct cell populations at different time points or to assess stage-specific roles of genes of interest. In fact, the *Sox10-iCreER^{T2}* line by Laranjeira and colleagues has been used to show that glia in the adult enteric nervous system are able to produce neurons upon injury (Laranjeira et al., 2011) or that cells in peripheral nerves can give rise to parasympathetic neurons, dental mesenchymal cells, or neuroendocrine chromaffin cells in the adrenal medulla (Dyachuk et al., 2014; Furlan et al., 2017; Kauka et al., 2014). Such studies might also be possible with yet another independently generated *Sox10-iCreER^{T2}* line (McKenzie et al., 2014) or with a *Sox10ER^{T2}CreER^{T2}* line termed *SECE* (He & Soriano, 2015). Of note, while high dose TM treatment in *SECE/R26R* embryos resulted in labeling of multiple neural and nonneural neural crest derivatives, low dose application of TM was found to affect the reporter gene expression pattern, allowing the tracing specifically of cranial as opposed to trunk neural crest cells (He & Soriano, 2015).

Another genetic tool suitable for neural crest cell fate mapping at different developmental stages is a *Plp-CreER^{T2}* mouse line generated by Leone and colleagues (Leone et al., 2003). *Plp* is a glia-specific

marker, and the *Plp* gene regulatory elements used to drive *CreERT2* expression in these mice were reported to direct specific transgene expression in oligodendrocytes and Schwann cells. Accordingly, TM-induced recombination during embryonic development and in adult mice led to very efficient labeling of peripheral glia in *PLP-CreERT2/R26R* double transgenic animals (approximately 80% upon TM injection of adult mice) (Leone et al., 2003). Moreover, using this line, an unexpectedly broad developmental potential of peripheral glial cells was revealed, with *Plp-CreERT2*-traced cells along peripheral nerves giving rise to cell types as diverse as melanocytes, parasympathetic neurons, mesenchymal cells in teeth, and chromaffin cells of the adrenal medulla (Adameyko et al., 2009; Dyachuk et al., 2014; Furlan et al., 2017; Kauka et al., 2014; Petersen & Adameyko, 2017). The glial origin of at least some melanocytes was also suggested by fate mapping experiments using another inducible *Plp-Cre*-driver line, *Plp-CreER* (Doerflinger, Macklin, & Popko, 2003). Using this line, it was reported that melanocytes in hair follicles, but not in the interfollicular epidermis of the tail, originate from *Plp-Cre*-positive glial cells at E11.5 in the mouse (Deo, Huang, Fuchs, de Angelis, & Van Raamsdonk, 2013). The potential of peripheral glia to generate nonglial cell types was also demonstrated by alternative approaches, involving fate mapping with other lineage-specific Cre-driver lines or single cell RNA sequencing (Espinoza-Medina et al., 2014; Furlan et al., 2017; Uesaka, Nagashimada, & Enomoto, 2015). The latter allowed generation of “pseudo-time” lineage trajectories and revealed an intermediate cellular state in between the states defining Schwann cell precursors and differentiated nonglial cells, respectively (Furlan et al., 2017). Finally, the *Plp-CreERT2* line (Leone et al., 2003) was instrumental to demonstrate by fate mapping that adult peripheral glia become activated upon skin wounding, detach from axons, and colonize the wound bed to support wound healing in a paracrine manner, without notable differentiation into other, nonglial cell types (Parfejevs et al., 2018).

However, inducible Cre activity in the *Plp-CreERT2* line turned out not to be specific for the glial lineage (Hari et al., 2012; Leone et al., 2003). Indeed, in *PLP-CreERT2/R26R* embryos, TM treatment at early stages of neural crest development (E9.5) resulted in prominent labeling of peripheral neurons, glia, and melanocytes, i.e., a fate map highly reminiscent of multipotent neural crest cells (Hari et al., 2012). The expression pattern of β -galactosidase became gradually restricted upon TM injection at later stages. However, at all stages examined, the melanocytic lineage was marked with considerable efficiency upon *PLP-CreERT2*-mediated recombination. Likewise, induction of *PLP-CreERT2*-driven recombination in adult mice marked a substantial fraction of skin melanocytes, independently of the Cre-reporter allele used (Parfejevs et al., 2018). Some of this expression might be due to *CreERT2* leakiness during early neural crest development, given that in adult skin of both *PLP-CreERT2/R26R-tdTomato* and *Sox10-iCreERT2/R26R-tdTomato* mice, about 25% of all hair follicles contain recombined melanocytes even in the absence of any TM treatment (Figure 2) (Parfejevs and Sommer, unpublished). However, TM injection in the adult significantly induced Cre-reporter expression in both peripheral glia and melanocytes in the skin (Parfejevs et al., 2018), demonstrating persistent activity of *PLP-CreERT2* and *Sox10-iCreERT2* in these adult tissues.

Thus, as with *Sox10-CreERT2*-expressing mice, different neural crest derivatives can be traced by means of the *PLP-CreERT2*-line, albeit with an apparently lower recombination efficiency. Recently, Kauka and colleagues made use of this feature to carry out clonal analysis of cranial neural crest cells in *PLP-CreERT2/R26R-Confetti* embryos to confirm data obtained with a *Sox10-iCreERT2* line (Kauka et al., 2016).

In conclusion, distinct genetic mouse lines are available for fate mapping premigratory and migratory neural crest cells. Together with Cre-driver lines specific for fate-restricted precursor cells that we have not covered in the present review, there is an increasing tool set available to the community to study the neural crest lineage tree and the molecular mechanisms shaping it. The finding that several Cre and *CreERT2* driver lines expected to exhibit lineage-specific expression appear to mark multipotent neural crest cells (although with quite divergent recombination efficiencies) could simply reflect unfaithful transgene expression. Alternatively, however, migratory neural crest cells as well as, for instance, cells in peripheral nerves might comprise distinct subpopulations expressing supposedly lineage-specific markers together with multipotency markers. Conceivably, such cell populations may be more or less ready to respond to the activity of cues controlling fate decisions during development or upon injury. To address such issues, the genetic approaches for prospective lineage tracing of neural crest cells described herein will have to be complemented with other methods, notably including retrospective lineage tracing by single cell transcriptome analysis.

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