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Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice

Clare L. Abram, Gray L. Roberge, Yongmei Hu, Clifford A. Lowell *

Department of Laboratory Medicine and the Program in Immunology, University of California, San Francisco, CA 94143, USA

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ABSTRACT

Since the first example of conditional gene targeting in mice in 1994, the use of Cre recombinase and loxP flanked sequences has become an invaluable technique to generate tissue and temporal specific gene knockouts. The number of mouse strains expressing floxed-gene sequences, and tissue-specific or temporal-specific Cre-recombinase that have been reported in the literature has grown exponentially. However, increased use of this technology has highlighted several problems that can impact the interpretation of any phenotype observed in these mouse models. In particular, accurate knowledge of the specificity of Cre expression in each strain is critical in order to make conclusions about the role of specific cell types in the phenotypes observed. Cre-mediated deletion specificity and efficiency have been described in many different ways in the literature, making direct comparisons between these Cre strains impossible. Here we report crossing thirteen different myeloid-Cre mouse strains to ROSA-EYFP reporter mice and assaying YFP expression in a variety of naïve unstimulated hematopoietic cells, in parallel. By focusing on myeloid subsets, we directly compare the relative efficiency and specificity of myeloid deletion in these strains under steady-state conditions.

1. Introduction

When Sauer et al. described site-specific DNA recombination using Cre recombinase in 1988, they suggested that this "may be a useful tool for understanding and modulating genome rearrangements in eukaryotes" (Sauer and Henderson, 1988). Directly repeating 34 base pair loxP DNA sequences are placed flanking a target gene ("floxed"). Expression of Cre recombinase in the same cell leads to specific deletion of the floxed sequence. These observations were soon extended to show that site-specific deletion could be achieved in transgenic mice (Lakso et al., 1992; Orban et al., 1992), and by restricting Cre expression to a particular cell type, tissue specific gene deletion was demonstrated (Gu et al., 1994). Twenty five years later, this technology has become an invaluable tool used in laboratories for designing mouse models to answer a variety of research questions, especially in cases where complete gene knockouts cause embryonic or perinatal lethality. Use of inducible promoters that express Cre recombinase upon addition of agents like tamoxifen, tetracycline or type I interferon has enabled temporal analysis of gene function without complications caused by gene deletion during development. Inducible expression of an exogenous gene by inserting a *lox-STOP-lox* sequence upstream of a transgene has further expanded applications of this technology to include methods such as cell type-specific deletion mediated by diphtheria toxin, and lineage tracking mediated by expression of markers such as beta galactosidase or EYFP (Srinivas et al., 2001; Brockschnieder et al., 2006). In addition to the generation of many floxed mouse

Abbreviations: EYFP, enhanced yellow fluorescent protein; HBSS, Hanks balanced salt solution; BAL, bronchioalveolar lavage; BAC, bacterial artificial chromosome; IRES, internal ribosome entry site; DC, dendritic cell; NK, natural killer.

* Corresponding author at: Department of Laboratory Medicine, University of California, San Francisco, 513 Parnassus Avenue, MSB-1058, CA 94143-0451, USA. Tel.: +1 415 476 2963; fax: +1 415 502 5462.
E-mail address: Clifford.Lowell@ucsf.edu (C.A. Lowell).

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strains, there has been a huge increase in the generation of Cre-expressing mouse strains, including several large-scale efforts to generate (predominantly neural-focused) new strains and resources to track them, comprehensively reviewed in Smedley et al. (2011) and Murray et al. (2012).

Not surprisingly, as the use of this technology, once described as the "Universal reagent for genome tailoring" (Nagy, 2000), has expanded, several issues have arisen that researchers must be aware of in interpreting results from these mouse models, reviewed in Schmidt-Supprian and Rajewsky (2007). In particular, the specificity of Cre expression is especially important but publications frequently fail to include comprehensive Cre expression profiles across many cell types. There are several methods for generating Cre-expressing strains, using either a transgene that includes a specific promoter or a "knock in" approach that uses endogenous regulatory sequences. Off target effects can arise from unexpected gene deletion caused by ectopic Cre expression or loss of enhancers or repressors that affect promoter activity. Examples of unexpected Cre expression in mice used for lymphoid cell analysis include non hematopoietic cells and germline expression (Schmidt-Supprian and Rajewsky, 2007). Unexpected expression of Cre in the germline can lead to passage of the deleted gene on to subsequent generations, so breeding strategies used for generating these mouse models must be carefully regulated. Use of bacterial artificial chromosomes (BACs) to generate a BAC transgenic that includes more regulatory sequences, or utilizing a neutral docking site that reduces transgene insertion site variation can improve these issues. A knock-in approach using the endogenous locus can be an advantage, although loss of one gene copy can lead to hemizygous effects. Newer lines have incorporated Internal Ribosome Entry Site (IRES)-Cre cassettes, leaving the regulatory gene intact. Although expression of Cre recombinase does not seem to affect mouse development, it has been suggested that at high concentrations, Cre can mediate DNA damage (Schmidt et al., 2000). This might be occurring through pseudo *loxP* sites (Thyagarajan et al., 2000; Semprini et al., 2007). The *RIP-cre* line was found to develop glucose intolerance in the absence of *loxP* targeted genes (Lee et al., 2006) and other examples of Cre toxicity have been reported in the gut and immune cell compartments (Higashi et al., 2009; Huh et al., 2010). Maintaining control of *cre* copy number in transgenic strains when designing breeding strategies may reduce this. Other factors that can influence deletion patterns include the genetic background of the Cre strain, and the sex of the parent contributing the *cre* allele, due to variation in Cre expression between the testes and ovary (Hebert and McConnell, 2000; Heffner et al., 2012). Furthermore, monitoring gene deletion by a PCR-based screen that detects just the *cre* allele can be inaccurate because silencing of this allele has been reported, perhaps due to methylation or other epigenetic changes (Schulz et al., 2007; Long and Rossi, 2009; Huh et al., 2010). Consequently, the presence of the deleted allele should also be monitored. Deletion efficiency using the same Cre strain can also vary depending on the floxed alleles or be inconsistent between littermates. These examples highlight the care that must be taken in analyzing data using these mouse models.

Many Cre strains are reported to be specific for certain cell types and tissues, and data is provided to indicate specificity, but a complete expression pattern is often not reported. As more strains become available, it is useful to be able to compare these different strains in a standard way and determine their relative specificity. Jackson Laboratories has undertaken the development of a Cre portal (www.creportal.org) in order to provide researchers with high throughput data about different Cre strains (Heffner et al., 2012). This work in progress presents a very thorough, histological analysis using beta-galactosidase from four stages of mouse development (E10.5, E15.5, P7 and P56). Our report complements this work, providing a more detailed approach looking at the specificity of Cre expression in myeloid-Cre-driven strains, using a standard protocol against which other myeloid-Cre strains could be compared in the future. We have used a *ROSA-flox-stop-flox*-EYFP reporter mouse to assay the expression patterns of Cre recombinase side-by-side in thirteen myeloid-Cre strains by flow cytometry. Such a parallel comparison using a standardized method has not been reported previously and should provide a useful resource to researchers to guide experimental design.

2. Methods

2.1. Mice and reagents

GE-cre, *LysM-cre*, *MRP8-cre*, *CD11c-cre*, *Vav1-cre*, *F4/80-cre*, *PF4-cre*, *CD11b-cre*, *NKp46-cre*, *CMA1-cre*, *MCPT5-cre*, *Basoph8-*

Table 1
Primers used for PCR genotyping.

Mouse strain	Primers used for genotyping
GE-cre	F = 5′ CAT GAC ACC CCC ACT GTC GTG TCC R (wt) = 5′ CAA TGC CAG TAG CAT GGC AGC CAG R (cre) = 5′ CAG GTA ATC TCT CAC ATC CTC AGG
LysM-cre	F = 5′ CTT GGG CTG CCA GAA TTT CTC R (wt) = 5′ TTA CAG TCG GCC AGG CTG AC R (cre) = 5′ TCA GCT ACA CCA GAG ACG G
MRP8-cre, CD11b-cre and CMA1-cre	F = 5′ CTG CAT TAC CGG TCG ATG CAA C R = 5′ GCA TTG CTG TCA CTT GGT CGT G
CD11c-cre	F = 5′ ACT TGG CAG CTG TCT CCA AG R = 5′ GCG AAC ATC TTC AGG TTC TG
Vav1-cre	F = 5′ AGA TGC CAG GAC ATC AGG AAC CTG R = 5′ ATC AGC CAC ACC AGA CAC AGA GAT C
F4/80-cre	F = 5′ AGA GGA GCA GCC AAA AGC CCC R (wt) = 5′ CTG ATG GTG GCA ACT CAG C R (cre) = 5′ GCG AAC ATC TTC AGG TTC TG
PF4-cre	F = 5′ CCC ATA CAG CAC ACC TTT TG R = 5′ TGC ACA GTC AGC AGG TT
NKp46-cre	F = 5′ GGA ACT GAA GGC AAC TCC TG R (wt) = 5′ TTC CCG GCA ACA TAA AAT AAA R (cre) = 5′ CCC TAG GAA TGC TCG TCA AG
MCPT5-cre	F = 5′ ACA GTG GTA TTC CCG GGG AGT GT R = 5′ GTC AGT GCG TTC AAA GGC CA
Basoph8-cre	F (wt) = 5′ GCT CTT CCA CCT CCT CAG TG F (cre) = 5′ CCA GCC ATC TGT TGT TTG C R = GGG ATG AGG ATG GTT GCT TA
Cx3cr1-cre	F = 5′ ACG TGG ACC TGC TTA CTG CAT G R = 5′ CGG CAA ACG GAC AGA AGC ATT
ROSA26-YFP	F = 5′ AAA GTC GCT CTG AGT TGT TAT R (wt) = 5′ GCG AAG AGT TTG TCC TCA ACC R (KI) = 5′ GGA GCG GGA GAA ATG GAT ATG