Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus

ABSTRACT

In contrast to existing lacZ reporter lines, where lacZ expression cannot easily be detected in living tissue, the EYFP and ECFP reporter strains are useful for monitoring the expression of Cre and tracing the lineage of these cells and their descendants in cultured embryos or organs. The non-overlapping emission spectra of EYFP and ECFP make them ideal for double labeling studies in living tissues.

INTRODUCTION

Background The Cre-loxP site specific recombination system is widely used for production of tissue-specific and conditional knockout alleles in mice. Recently, a Cre-dependent lacZ reporter strain (R26R) was produced by targeted insertion of a lacZ gene, preceded by a loxP-flanked (floxed) strong transcriptional termination sequence (tpA), into the ubiquitously expressed ROSA26 locus. The R26R allele terminates transcription prematurely, but when the mice are crossed with Cre-expressing transgenic mice, the Cre-mediated excision of the floxed termination sequence leads to constitutive lacZ expression. Thus, these doubly transgenic animals express lacZ only in the cells that have expressed Cre, as well as in all of their daughter cells. Similar Cre reporter strains have been produced using different promoters to express lacZ [], Another variation on this theme has been the development of the Z/AP reporter strain, which switches from lacZ to alkaline phosphatase expression upon exposure to Cre recombinase activity. All of these strains are useful for monitoring the expression of Cre recombinase, as well as for cell lineage tagging experiments [,]. Here, we describe the production of two similar Cre reporter alleles that express enhanced yellow or cyan fluorescent protein (EYFP or ECFP), two color variants of the green fluorescent protein (GFP). GFP and its variants are autofluorescent proteins that can be visualized in living cells, and are therefore particularly useful for monitoring gene expression in whole embryos, animals or cultured cells and organs. EYFP and ECFP were chosen because their emission spectra overlap minimally, so they can be distinguished when used simultaneously, whereas the emission spectra of EYFP and ECFP overlap to a greater extent with that of EGFP http://www.clontech.com/gfp/pdf/LivingColors.pdf. During the course of this work, three new Cre reporter strains that conditionally express EGFP were reported. Two transgenic strains use the β-actin promoter/CMV enhancer to express EGFP following Cre-mediated excision of a stop sequence, and in one of these, lacZ is expressed prior to the Cre-mediated excision event. In the third strain, which is similar in principle to the YFP and CFP alleles reported here, EGFP was inserted at the ROSA26 locus. The availability of different Cre reporter strains will be valuable, not only because of the advantages of different reporter proteins, but also because the efficiency of Cre-mediated excision may be dependent on the target locus.

CONCLUSION

Conclusions We have constructed two reporter lines of mice that express EYFP or ECFP only in cells expressing the Cre recombinase, and their daughter cells, by targeting these cDNAs into the ubiquitously expressed ROSA26 locus, preceded by a loxP flanked "stop" sequence. Crosses with a general Cre expressing strain (βactin-Cre) and two tissue-specific Cre

strains (Isl1-Cre and En1-Cre) showed that the reporter strains function as expected, based on their similarity to the well characterized R26R-lacZ strains. In contrast to lacZ expression, which cannot be easily detected in living tissue, the EYFP and ECFP reporter strains (together with the GFP reporter strains currently available) will be very useful for monitoring the expression of Cre in living tissues, or tracing the lineage of these cells and their descendants, in cultured embryos or organs. Furthermore, by using modified forms of Cre whose recombinase activity is inducible, one can use these reporter mice to perform detailed analysis on the lineage of cells at different time points during development.