Ubiquitous Expression of mRFP1 in Transgenic Mice

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Summary: Fluorescent proteins provide a powerful means to track gene expression and cellular behaviors in the study of model organisms such as mice. Among the new generation of fluorescent protein markers, the monomeric red fluorescent protein mRFP1 is particularly attractive because of its rapid maturation and minimal interference with GFP and GFP-derived markers. Here we evaluate the utility of mRFP1 as a marker in transgenic mice. We show that high level and ubiquitous expression of mRFP1 does not affect mouse development, general physiology, or reproduction. mRFP1 expression can be readily detected with unaided eyes under daylight in transgenic mice on the albino background. The intensity of mRFP1 signals can be used to distinguish homozygous and heterozygous transgenic mice. Together, these features make mRFP1 an attractive marker for broad applications in transgenic research.

Key words: RFP; transgenic marker; FVB; sperm; oocyte

Reporter genes are widely used to follow gene expression and cellular behavior in development and disease studies in mice. For instance, the ubiquitous expression of a LacZ reporter in the ROSA 26 mouse has made this strain a great tool for chimera and transplantation studies (Zambrowicz et al., 1997). However, the need for sample fixation and substrate permeation are incompatible with its detection in living cells and live animals. This problem can be overcome by the use of fluorescent reporters such as enhanced green (EGFP) (Okabe et al., 1997), yellow (EYFP), and cyan (ECFP) fluorescent proteins (Srinivas et al., 2001), which allow for detection of reporter expression in living cells or animals (Yang et al., 1999). The red fluorescent proteins (RFP) represent a different type of fluorescent proteins, initially isolated as DsRed from a sea anemone relative, Discosoma (Matz et al., 1999). DsRed matures slowly and forms tetramers in vivo and thus its application as a transgenic marker is limited in comparison with GFP and GFP derivatives (Baird et al., 2000; Hadjantonakis et al., 2002). mRFP1 is a quick-maturing monomeric variant of DsRed with more red-shifted emission and excitation spectrum (Campbell et al., 2002). In search of a robust transgenic marker, we investigated the applicable features of mRFP1 in transgenic mice and conclude that mRFP1 is not only an alternative marker to GFP but also unique in many applications, including its easy detection without a fluorescent light source.

Transgenic mice were generated by pronuclear coinjection of linearized pCX-mRFP1 DNA with unrelated transgenes. Founders were selected after PCR genotyping with a pair of primers flanking the mRFP1 coding sequence. A total of 21 PCR-positive founders was established, all of which transmitted the mRFP1 transgenes to their offspring. Transgenic lines were examined under a portable longwave UV lamp to judge the expression level of mRFP1. Eighteen lines out of the 21 founders showed red fluorescence under UV illumination. In fact, most of them were visibly red in daylight (Fig. 1a). We chose one pCX-mRFP1 transgenic line that shows moderate color intensity for subsequent analysis. This transgenic line also carries a separate transgene, which is independently regulated from the coinjected pCX-mRFP1 (data not shown).

We first addressed the question of whether the fluorescent intensity of mRFP1 correlates with gene dosage.
Due to the position effect on individual transgenes, it is difficult to compare the relationship between mRFP1 copy numbers and its expression levels in different transgenic lines. Thus, we doubled the copy number by self-crossing heterozygous pCX-mRFP1 mice. The offspring resulting from such a cross could be easily separated into three groups according to the intensity of red fluorescence. We termed these groups double red, red, and non-red (Fig. 1b). To determine if mice of the double red group were homozygotes while mice of the red group were heterozygotes, each adult male mouse of these two groups was crossed with wildtype FVB/NJ females and the genotype of their offspring were determined by PCR. As expected, all offspring (17/17) of double red males carried the pCXmRFP1 transgene. While about half of the offspring (9/20) of red males were pCX-mRFP1-positive, transgenic positive offspring from both groups showed similar levels of red fluorescence. The correlation between red fluorescent intensity and gene dosage was also observed in several other transgenic lines carrying pCX-mRFP1.

We next determined the expression pattern of the pCX-mRFP1 transgene in tissues and cells taken from adult transgenic heterozygous mice. Red fluorescence was detected in tissue samples including brain, heart, liver, kidney, testis, lung, muscle, intestine, thymus, spleen, seminal vesicle, skin, hair, fat, and blood cells isolated from the transgenic positive mice but not in control littermates (Fig. 2 and data not shown). Under brightfield illumination, most organs of transgenic mice are red colored and readily distinguished from those of wildtype mice (Fig. 2). The level of red fluorescence was strong in most of the tissues checked, but relatively weak in fat, lung, spleen, testis, and absent in red blood cells.

The expression of pCX-mRFP1 transgene can be detected as early as the haploid sperms and oocytes (Fig. 3). We also examined the onset of RFP expression during the development of preimplantation stage embryos. In the cross between the transgenic males and wildtype females, we show that somatic RFP expression begins at the blastocyst stage (Fig. 3b,c). In the cross between the transgenic females and wildtype males, RFP expression was detected throughout the development of preimplantation stage embryos. However, the maternal effect of gene expression and somatic gene expression cannot be easily distinguished in this type of cross (Fig. 3d,e).
Despite a significant amount of mRFP1 expression throughout the body, including brain and muscle, pCX-mRFP1 transgenic mice were apparently normal and showed similar fertility, gross behavior, and lifespan as wildtype FVB/NJ mice. Analysis of body weight and length and the size of organs, including heart, liver, kidney, and spleen, revealed no significant difference between age- and sex-matched pCX-mRFP1 transgenic mice and wildtype mice (n = 3 for each genotype group, data not shown). We further evaluated neural and muscular functions of the transgenic mice in the Rotarod test. In this assay mice were tested for their ability to...
mRFP1 expression in germ cells and preimplantation embryos. Half of the sperm collected from heterozygous pCX-mRFP1 transgenic male mice expressed mRFP1 (a–a'). The red fluorescence was absent in two-cell and morulae stage embryos (b–b') and present in half of the blastocysts (c–c') derived from a mating between heterozygous transgenic male and FVB/NJ females. When homozygous pCX-mRFP1 transgenic female mice were crossed with wildtype FVB/NJ males, mRFP1 expression could be observed in oocytes (d–d') and throughout preimplantation development (e–e'). a–e: Photos taken with a DsRed filter. a'–e': The same samples observed under brightfield. a'–e': Merged images.
Transgenic mice were produced by microinjecting the bor, ME) and bred in a clean facility at Fudan University. Originally purchased from Jackson Laboratories (Bar Harbor, ME). All transgenic mice were produced with FVB/NJ mice, which were excised with BamHI and SalI and gel-purified.

**Construction of Transgenic Vectors**

The coding sequence (CDS) of mRFP1 was cut from pRSETb-mRFP1 (Campbell et al., 2002) by BamHI and EcoRI digestion, treated with Klenow, and used to replace the EGFP CDS between two EcoRI sites of pCX-mRFP1 (Okabe et al., 1997). The resulting plasmid, named as pCX-mRFP1, contains a CMV enhancer, chicken beta-actin promoter, mRFP1 CDS, and rabbit beta-globin polyA site. The entire mRFP1 expression unit was excised with BamHI and SalI and gel-purified.

**Production of Transgenic Mice**

All transgenic mice were produced with FVB/NJ mice, originally purchased from Jackson Laboratories (Bar Harbor, ME) and bred in a clean facility at Fudan University. Transgenic mice were produced by microinjecting the purified BamHI and SalI fragment of pCX-mRFP1 with linearized transgenes into fertilized FVB/NJ eggs. Transgenic founders were identified by PCR with primers PCXF (5'-CTTTTTATGGTAATCGTGCGAGAG-3') and Act-pAB (5'-CTGATAGGCAGCCTGCACCTGAGGAGTG-3'). Founders were mated with FVB/NJ mice to produce transgenic lines.

**Detection of Red Fluorescence**

Red fluorescence of living animals was detected using a portable longwave UV light (Furi, Shanghai). Photos of fluorescent microscopy were taken with Leica microscopes equipped with Texas Red or DsRed filters.

**Mouse Behavioral Test**

Heterozygous transgenic mice and their wildtype littermates of the same sex were used in the behavioral test. The Rotarod assay was performed as previously described (Dunham and Miya, 1957). Mice at 8 weeks of age were given three trials per day for 5 consecutive days. Each trial last for 5 min, during which mice were placed onto a rod with a diameter of 87 mm that rotated at a speed of 10 rpm. The retention time was scored as the length of period that an animal successfully kept itself on the rod. The test was stopped after 5 min.

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**LITERATURE CITED**


