Journal of Neuroscience Methods 196 (2011) 88–98

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

OMP-ZsGreen fluorescent protein transgenic mice for visualisation of olfactory sensory neurons in vivo and in vitro

Jenny A.K. Ekberg[a], Daniel Amaya[a], Fatemeh Chehrehasa[a], Katie Lineburg[a], Christina Claxton[b], Louisa C.E. Windus[a], Brian Key[b], Alan Mackay-Sim[a], James A. St John[a,*]

[a] National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, 170 Kessels Road, Griffith University, Nathan 4111, Brisbane, QLD, Australia
[b] School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form
29 December 2010
Accepted 3 January 2011

Keywords:
Axon
Olfactory marker protein
Glia
Fascicle
Olfactory bulb
Olfactory ensheathing cell

ABSTRACT

Research into the biology of the mammalian olfactory system would be greatly enhanced by transgenic reporter mice with cell-specific fluorescence. To this end we previously generated a mouse whose olfactory ensheathing cells (OECs) express DsRed driven by the S100ß promoter. We present here a transgenic reporter mouse whose olfactory sensory neurons express ZsGreen, driven by the olfactory marker protein (OMP) promoter. ZsGreen was very strongly expressed throughout the cytoplasm of olfactory sensory neurons labelling them in living cells and after fixation. Labelled sensory neurons were seen in all olfactory regions in the nose and fluorescent axons coursed through the lamina propria and into the main and accessory bulbs. We developed methods for culturing embryonic and postnatal olfactory sensory neurons using these mice to visualise living cells in vitro. ZsGreen was expressed along the length of axons providing exceptional detail of the growth cones. The ZsGreen fluorescence was very stable, without fading during frequent imaging. The combination of OMP-ZsGreen and S100ß-DsRed transgenic mice is ideal for developmental studies and neuron–glia assays and they can be bred with mutant mice to dissect the roles of various molecules in neurogenesis, differentiation, axon growth and targeting and other aspects of olfactory sensory neuron and glia biology.

1. Introduction

The mammalian olfactory nervous system is characterised by a complex topographic map. Within the olfactory epithelium that lines the nasal cavity, primary sensory neurons that express the same odorant receptor are mosaically distributed, yet their axons terminate in 1–2 glomeruli in topographically fixed positions in the olfactory bulb within the central nervous system (Mombaerts et al., 1996; Royal and Key, 1999). Unlike the visual system, the axons of olfactory sensory neurons do not maintain a near-neighbour relationship but instead intermingle within the olfactory nerve (Mombaerts et al., 1996). Evidence to date indicates that the establishment of the olfactory topographic map involves the dynamic and coordinated expression of a range of guidance molecules, an intimate interactions between the axons and the glia of the olfactory nerve, the olfactory ensheathing cells (OECs) as well as the formation of synaptic connections with second order neurons and myriad interneurons (see reviews by Cho et al., 2009; Imai and Sakano, 2009).

While most of our understanding of axon guidance in the visual system was initially determined by in vitro axon outgrowth assays, axon guidance in the olfactory system has typically been assessed by examining phenotypes observed in genetically engineered knock-in and knock-out mice (e.g. St John et al., 2006; Takeuchi et al., 2010; Treloar et al., 2009). This approach has dominated because there is a lack of robust in vitro assays to investigate the role of olfactory axon guidance molecules in the olfactory system. Analysing the role of selected molecules that modulate the growth of primary olfactory axons in vitro, in particular from postnatal neurons, is complicated by the requisite need for the presence of OECs for olfactory axon growth (Tisay and Key, 1999).

Intense research is being undertaken into the biology and function of the different cells within the main olfactory system as well as comparisons with cells from different sensory organs in the nose including the accessory olfactory system, the organ of Masera (Pedersen and Benson, 1986; Weiler and Farbman, 2003) and the septal organ of Grüneberg (Roppolo et al., 2006; Storan and Key, 2006). The unambiguous identification of living neurons and glia in vivo and in vitro would greatly facilitate the study of neuron–glia interactions, calcium imaging, electrophysiology experiments and the development of axon guidance and cell behaviour assays.

* Corresponding author. Tel.: +61 7 3735 3660; fax: +61 7 3735 4255.
E-mail address: j.stjohn@griffith.edu.au (J.A. St John).

0165-0270/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.01.008

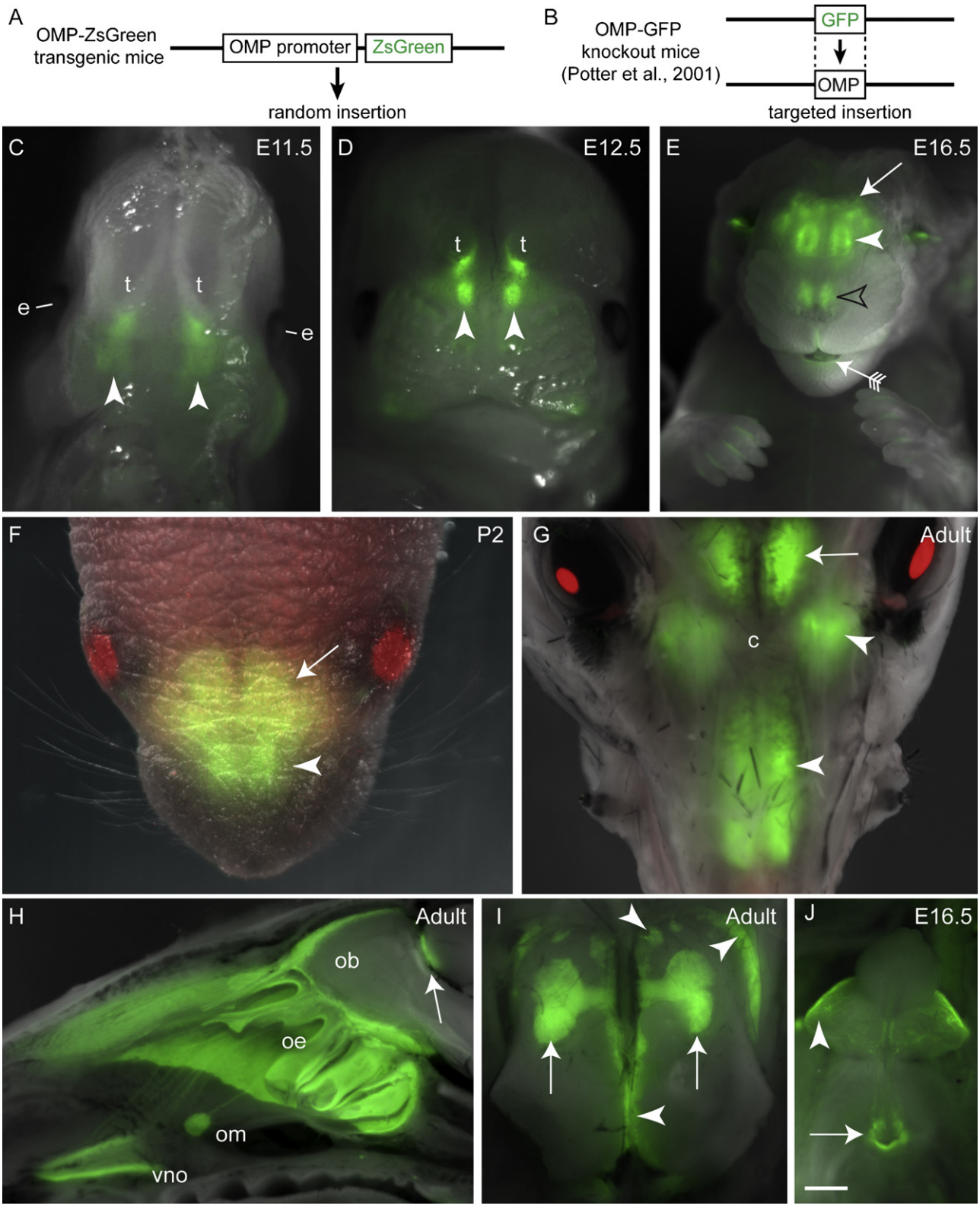

Fig. 1. OMP-ZsGreen expression in transgenic mice. (A) The OMP-ZsGreen transgene consisted of the full length 5.5 kb OMP promoter sequence which drove expression of the ZsGreen coding sequence. The mice were generated by random insertion into the genome. (B) The previously generated OMP-GFP knockout mice (Potter et al., 2001) were generated by targeted insertion of the GFP coding sequence which replaced the coding sequence of OMP. Panels C–J show wholemount preparations of OMP-ZsGreen transgenic mice. (C) In a frontal view of an E11.5 embryo, ZsGreen expression was visible in the olfactory epithelium of the nasal cavity (arrowheads); t is telencephalon, e is eye. (D) In E12.5 embryos, ZsGreen expression was visible in the nasal cavity (arrowheads) as well as lining the medial regions of the telencephalon of the presumptive olfactory bulb (t). (E) In E16.5 embryos, ZsGreen expression was widespread through the nasal cavity (arrowhead) and olfactory bulbs (arrow) as well as in the septal organ of Grüneberg (unfilled arrowhead). ZsGreen was ectopically expressed in the oral cavity (arrow with tail) and in the margins of the eyelids. (F) In a dorsal view of a living P2 OMP-ZsGreen × S100ß-DsRed mouse, the ZsGreen fluorescence in the nasal cavity (arrowhead) and olfactory bulb (arrow) was clearly visible through the skin. DsRed fluorescence was strongly expressed in the eyes. (G) In paraformaldehyde-fixed adult OMP-ZsGreen × S100ß-DsRed mouse with the skin removed, the ZsGreen fluoresence was visible in the nasal cavity (arrowheads) and olfactory bulbs (arrow), although fluorescence was not visible through the bony cribiform plate (c). (H) In a sagittal view