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Trpc2-expressing sensory neurons in the mouse main olfactory epithelium of type B express the soluble guanylate cyclase Gucy1b2

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ABSTRACT

Chemoreception in the mouse olfactory system occurs primarily at two chemosensory epithelia in the nasal cavity: the main olfactory epithelium (MOE) and the vomeronasal epithelium. The canonical chemosensory neurons in the MOE, the olfactory sensory neurons (OSNs), express the odorant receptor (OR) gene repertoire, and depend on *Adcy3* and *Cnga2* for chemosensory signal transduction. The canonical chemosensory neurons in the vomeronasal epithelium, the vomeronasal sensory neurons (VSNs), express two unrelated vomeronasal receptor (VR) gene repertoires, and involve *Trpc2* for chemosensory signal transduction. Recently we reported the discovery of two types of neurons in the mouse MOE that express *Trcp2* in addition to *Cnga2*. These cell types can be distinguished at the single-cell level by expression of *Adcy3*: positive, type A and negative, type B. Some type A cells express OR genes. Thus far there is no specific gene or marker for type B cells, hampering further analyses such as physiological recordings. Here, we show that among MOE cells, type B cells are unique in their expression of the soluble guanylate cyclase Gucy1b2. We came across Gucy1b2 in an explorative approach based on Long Serial Analysis of Gene Expression (LongSAGE) that we applied to single red-fluorescent cells isolated from whole olfactory mucosa and vomeronasal organ of mice of a novel Trcp2-IRES-taumCherry gene-targeted strain. The generation of a novel Gucy1b2-IRES-tauGFP gene-targeted strain enabled us to visualize coalescence of axons of type B cells into glomeruli in the main olfactory bulb. Our molecular and anatomical analyses define Gucy1b2 as a marker for type B cells within the MOE. The Gucy1b2-IRES-tauGFP strain will be useful for physiological, molecular, cellular, and anatomical studies of this newly described chemosensory subsystem.

1. Introduction

Since the discovery of expression of the Trpc2 cation channel in rat vomeronasal organ (VNO) (Liman et al., 1999), Trpc2 expression was thought to be restricted to VSNs in mouse. We recently challenged this belief by immunohistochemistry (IHC) with the Trpc2 antibody that was used in rat (Liman et al., 1999) and by in situ hybridization (ISH) (Omura and Mombaerts, 2014). We also generated a Trpc2-IRES-taulacZ knockin mouse strain (Omura and Mombaerts, 2014). We found that the mouse MOE actually abounds with Trpc2+ cells, from early stages in development throughout adulthood. We identified two types of Trpc2+ MOE cells, which we refer to as type A and type B cells. These cell types can be distinguished at the single-cell level by *Adcy3* expression: type A cells express *Adcy3*, and type B cells do not express *Adcy3*. The cell bodies of type A cells form a semicontinuous layer throughout the MOE just below the sustentacular cell layer. The cell bodies of type B cells reside within the lateral region of the MOE and are located at all positions along the basal-to-apical dimension of the MOE. One third of MOE cells labeled with *Olfr68/69* riboprobes are type A cells. We were unable to find ISH evidence of expression of the known chemosensory G-protein coupled receptor genes or signaling components in type B cells. Type A and type B cells appear to share only *Trpc2* expression with VSNs, and are thus not VSNs that are misplaced in the MOE.

Here, we describe a novel gene-targeted knockin mutation in the *Trpc2* locus, designed to coexpress Trpc2 with the red-fluorescent axonal marker taumCherry. We picked single taumCherry+ MOE cells from the lateral region of the MOE (type B cells) and carried out RT-PCR analyses. We confirm and extend our ISH observations that type B cells do not express OR or VR genes. Next we applied LongSAGE to single taumCherry+ cells, and came across the soluble guanylate cyclase *Gucy1b2*. We show by ISH and by IHC with a custom Gucy1b2 antibody that Gucy1b2 expression is specific to type B cells in the MOE. We counted ~16,000 *Gucy1b2*+ cells in the MOE of C57BL/6 mice at three weeks, and found that 97% of these cells are *Trpc2*+. In mice of a novel gene-targeted Gucy1b2-IRES-tauGFP knockin strain, GFP+ axons coalesce into a few glomeruli ventrally and posteriorly in the main olfactory bulb. Our results thus define Gucy1b2 as a marker for type B cells in the MOE. The Gucy1b2-IRES-tauGFP strain will enable physiological experiments in type B cells to determine in which regard these cells differ functionally from type A cells, canonical OSNs, and VSNs.

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2. Results

2.1. The Trpc2-IRES-taumCherry knockin mouse strain

We have previously described a Trpc2-IRES-taulacZ knockin strain, in which Trpc2 + cells coexpress the axonal marker tauβgalactosidase (Omura and Mombaerts, 2014). This mouse strain lends itself well to X-gal histochemistry and IHC with βgalactosidase antibodies, but is less suited to study live, unfixed Trpc2 + cells. We therefore generated another mouse strain by gene targeting using the red fluorescent protein mCherry (Shaner et al., 2004). The genetic design of the Trpc2-IRES-taumCherry targeted mutation (Fig. 1A) mirrors that of Trpc2-IRES-taulacZ. The *IRES* sequence allows for cotranslation of intact Trpc2 polypeptide with the axonal marker taumCherry, and the

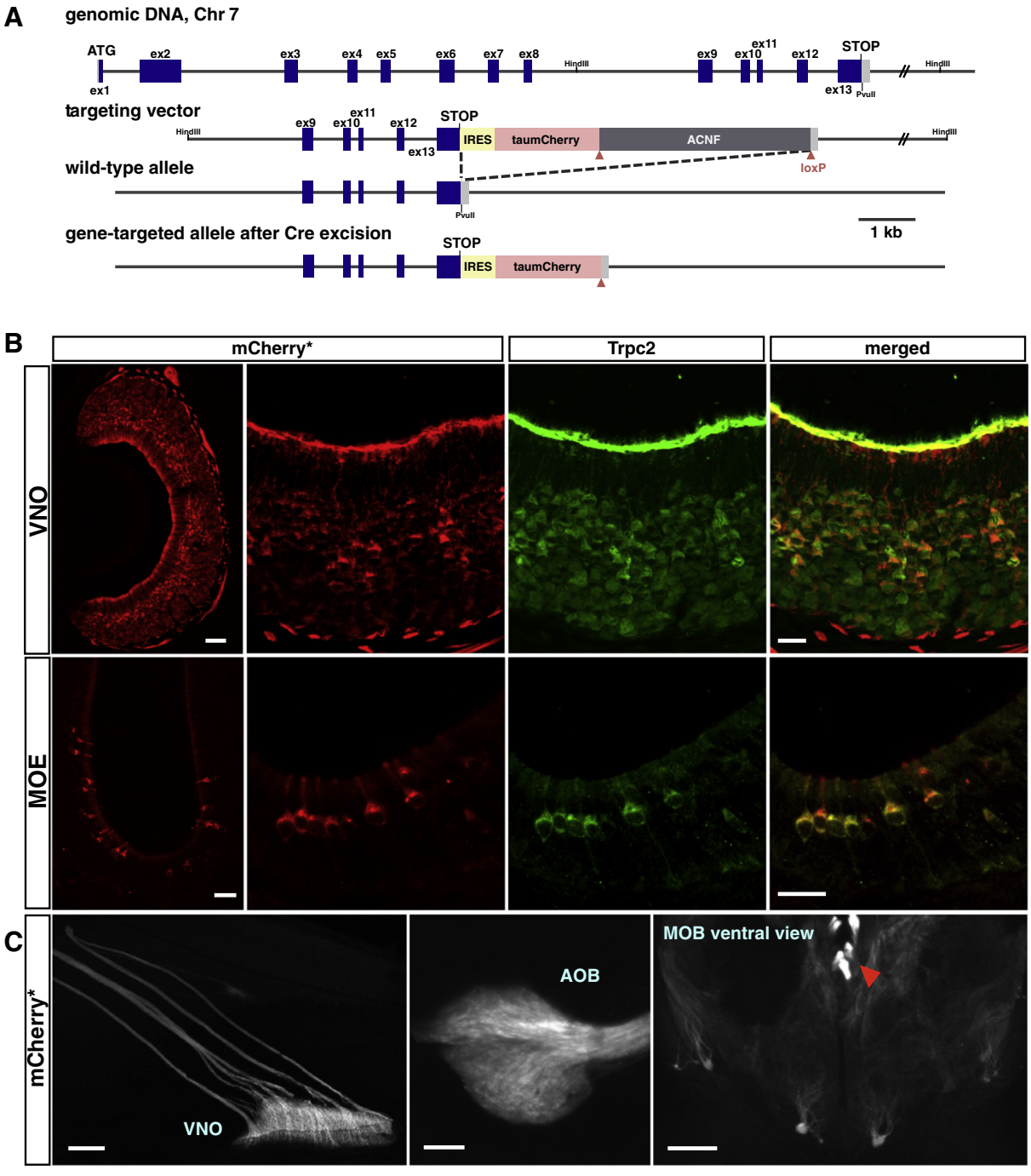

Fig. 1. Trpc2-IRES-taumCherry gene-targeted strain. (A) Generation of a Trpc2-IRES-taumCherry knockin mutation in the mouse germline. The *IRES-taumCherry-ANCF* cassette was inserted after the STOP codon of *Trpc2* by homologous recombination with a targeting vector in ES cells. The *ACNF* cassette is a self-excising *neo* gene that is removed during the transmission of the targeted allele through the male germ line, leaving a single *loxP* site (red triangle) behind in the locus. The axonal marker taumCherry is a fusion protein between bovine tau and mCherry and is intrinsically red fluorescent. (B) Intrinsic red fluorescence of taumCherry (mCherry*) of sections of the VNO and MOE of homozygous Trpc2-IRES-taumCherry mice at eight weeks, combined with IHC (green) for Trpc2. The fusion of mCherry to tau promotes subcellular localization at dendritic tips and axons compared to cell bodies. (C) Whole mount view of the olfactory system of a homozygous Trpc2-IRES-taumCherry mouse at four weeks, intrinsic red fluorescence (mCherry*). (Left) Axons project from the VNO across the nasal septum in the form of several vomeronasal fascicles. (Middle) Most axons terminate in the accessory olfactory bulb (AOB). (Right) Some axons coalesce into a few glomeruli ventrally in the main olfactory bulb (MOB). The red arrowhead indicates the vomeronasal nerve. Scale bar: 50 μm in B top left; 20 μm in B other panels; 500 μm in C left; 200 μm in C middle and right.