Molecular and Cellular Neuroscience 56 (2013) 212–224

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

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

Odorant receptor gene choice and axonal wiring in mice with deletion mutations in the odorant receptor gene *SR1*

Stefan H. Fuss [a,b], Yan Zhu [c,1], Peter Mombaerts [a,c,*]

[a] The Rockefeller University, New York, NY 10065, USA
[b] Bogazici University, Department of Molecular Biology and Genetics, 34342 Bebek, Istanbul, Turkey
[c] Max Planck Institute of Biophysics, Department of Molecular Neurogenetics, 60438 Frankfurt, Germany

ARTICLE INFO

Article history:
Received 14 March 2013
Revised 16 April 2013
Accepted 2 May 2013
Available online 18 May 2013

Keywords:
Odorant receptor
Gene choice
Gene regulation
Septal organ
Axon guidance
Glomerulus

ABSTRACT

In the mouse, a mature olfactory sensory neuron (OSN) of the main olfactory epithelium (MOE) expresses one allele of one of the 1200 odorant receptor (OR) genes in the genome. The mechanisms that underlie the one receptor–one neuron rule remain poorly understood. A popular experimental paradigm for OR gene choice is to delete an OR coding region by gene targeting or in a transgene. Here we have applied this ΔOR paradigm to *SR1*, also known as *MOR256-3* or *Olfr124*. This gene is expressed in OSNs of the MOE, and in ~50% of the OSNs of the septal organ. In heterozygous ΔSR1 mice, we observe an unprecedented biallelic expression rate of 30% at the *SR1* locus. In homozygous ΔSR1 mice, we find a significant increase in the number of septal organ OSNs that undergo apoptosis. As a population, ΔSR1 OSNs project their axons to 81–85 glomeruli in each half of the OB, and coexpress at least 77 OR genes as evaluated by single-cell molecular analysis. There are no obvious or simple rules for the set of OR genes that are coexpressed with the ΔSR1 allele. The frequencies of coexpression are different for ΔSR1 OSNs in the septal organ compared to those in the MOE. We propose that there are as many as five scenarios for the fate of individual ΔSR1 OSNs.

Introduction

Mature OSNs in the mouse are believed to express one allele of only one odorant receptor (OR) gene (Buck and Axel, 1991), from the repertoire of ~1200 intact OR genes that are scattered over >40 loci in the genome (Zhang et al., 2007). These features of monoallelic and monogenic expression (Fuss and Ray, 2009) endow OSNs with exquisite specificity of odorant recognition and anatomical connectivity. First, the expressed OR determines the odorant response profile of the OSN (Bozza et al., 2002; Malnic et al., 1999). Second, axons of OSNs expressing the same OR gene coalesce into a small number of glomeruli in the OB, and the expressed OR is a critical determinant of this axonal coalescence (Mombaerts et al., 1996). OR gene expression in the MOE is confined to one of multiple narrow expression domains, which are referred to as zones (Miyamichi et al., 2005; Ressler et al., 1993). The mechanisms of OR gene choice remain poorly understood. In particular the mechanisms that restrict expression of an OR gene to its zone remain elusive.

A popular experimental paradigm for understanding aspects of the mechanisms of OR gene choice is to delete or otherwise disable the OR coding region, either at the endogenous locus by gene targeting (Bozza et al., 2009; Feinstein et al., 2004; Grosmaitre et al., 2009; Lewcock and Reed, 2004; Shykind et al., 2004; Wang et al., 1998), or by conventional transgenesis at a random position in the genome (Imai et al., 2006; Serizawa et al., 2003; Vassalli et al., 2011). This design is referred to as ΔOR, reflecting the deletion of the OR coding region. A marker such as taulacZ or GFP is included in the targeted mutation or transgene in order to visualize the OSNs that express the ΔOR locus or transgene (ΔOR OSNs). The ΔOR design can be extremely precise – a clean deletion of the coding sequence from start to stop codon – because OR coding regions have no introns.

The first ΔOR study claimed that ΔOR OSNs have a reduced half-life, largely based on the observation that there were fewer labeled cells in older mice than in younger mice (Wang et al., 1998). This interpretation was later revised, and a transient nature of ΔOR expression in some ΔOR OSNs was invoked (Shykind et al., 2004). A consensus finding of the ΔOR studies is that a certain fraction of ΔOR OSNs coexpress a second OR locus with an intact coding region. In this experimental context, "coexpression" refers to the colocalization of marker RNA or protein produced from the ΔOR locus or transgene with RNA transcribed from any other endogenous OR locus in the same OSN. Consistent with coexpression of one of several other OR genes, ΔOR OSNs exhibit a variety of odorant response profiles (Feinstein et al., 2004; Grosmaitre et al., 2009). Another consistent finding of ΔOR studies is that axons of labeled ΔOR OSNs do not coalesce into a few glomeruli, but spread diffusely over a wide domain of the OB and enter many glomeruli.

* Corresponding author at: Max Planck Institute of Biophysics, Max-von-Laue-Strasse 3, D-60438 Frankfurt, Germany.
E-mail address: peter.mombaerts@biophys.mpg.de (P. Mombaerts).
[1] Present address: Key Laboratory of Contraceptive Drugs and Devices of National Population and Family Planning Committee, Shanghai Institute of Planned Parenthood Research, 200032 Shanghai, China.

1044-7431/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2013.05.002

The coexpression of an intact OR gene by an individual ΔOR OSN is classically interpreted by a cell-autonomous mechanism and as the consequence of the lack of negative feedback signaling within these cells. Normally the expressed OR would elicit a, still undefined, signal that somehow prevents expression of the other allele of the same OR gene and of all other OR genes in the genome (Serizawa et al., 2003). But in ΔOR OSNs, no OR protein is produced from the ΔOR locus or transgene, and hence, negative feedback does not occur. A certain percentage of ΔOR OSNs would then proceed to "choose" another OR gene for expression, and sometimes cease transcription of the ΔOR locus or transgene.

The temporal sequence of these transcription events (first ΔOR, then intact OR) has proved very difficult to demonstrate experimentally and convincingly; just one, indirect attempt has been reported, using Cre lineage tracing (Shykind et al., 2004). In most ΔOR studies the temporal sequence is only inferred or assumed. We therefore refer to expression of the intact OR gene in a ΔOR OSN as "coexpression" instead of the popular term "second choice"; this description is more neutral and reflects the experimental observations without assumptions. Thus, coexpression, as we here use this term, need not imply simultaneous transcription of the ΔOR locus with an intact OR gene.

The set of OR genes that are coexpressed in a population of ΔOR OSNs is restricted, and the rules defining this restriction remain elusive. Arguably, the set of coexpressed OR genes may represent the subset of the OR gene repertoire that is available for expression according to the MOE position, type, and/or lineage of the OSNs in question. The ΔOR paradigm thus offers a window into an intriguing aspect of OR gene choice: the subset of 1200 OR genes that are, in principle, available for expression to OSNs of a particular MOE position, type, and/or lineage.

Here, we have applied the ΔOR paradigm to an OR gene with an atypical expression pattern: *SR1*, also known as *MOR256-3* or *Olfr124*. This OR gene is expressed in OSNs in a ventral zone of the MOE, in a similar fashion as other OR genes that are expressed within this zone. In addition, *SR1* is expressed in ~50% of the OSNs in the septal organ (SO) (Kaluza et al., 2004; Tian and Ma, 2004, 2008). The two SOs of a mouse are small islands of olfactory epithelium that are located on both sides of the ventral nasal septum and are anatomically separated from the MOE. The cellular morphology and olfactory signal transduction of the OSNs in the SO (sOSNs) are indistinguishable from those of OSNs in the MOE (mOSNs) (Ma et al., 2003). Eight additional OR genes are expressed by another ~45% of sOSNs (Tian and Ma, 2008). SR1 is further atypical as an OR, in that SR1 sOSNs and mOSNs, and heterologous cells transfected with SR1 expression constructs, respond to a wide variety of chemical ligands and over a large range of concentrations (Grosmaitre et al., 2009).

We report a molecular and anatomical characterization of five mouse strains with targeted mutations in the *SR1* locus, four of which result in a neat deletion of the coding region (ΔSR1). We find that individual ΔSR1 OSNs coexpress one of at least 77 OR genes, and that their axons project to 81–85 glomeruli on each of the medial and lateral surfaces of the OB. The subset of OR genes that is available for coexpression is different for ΔSR1 sOSNs and mOSNs. We observe an unprecedented biallelic expression rate in sOSNs of heterozygous ΔSR1 mice, by three distinct methods: the *SR1* allele with the intact coding region is expressed in ~30% of ΔSR1 sOSNs in heterozygous ΔSR1 mice, but 10 times less frequently in ΔSR1 mOSNs. In homozygous ΔSR1 mice, a diverse and unlinked set of OR genes is available for coexpression, and the frequencies with which OR genes are coexpressed by ΔSR1 sOSNs and mOSNs are different. Thus, depending on whether a ΔSR1 OSN is located in the SO or in the MOE, a different subset of OR genes is available for coexpression.

Results

Gene targeting at the SR1 locus

Fig. 1 depicts the wild-type *SR1* locus and the five gene-targeted mutations that we have generated and characterized in this study. In the SR1-IRES-tauGFP strain (Grosmaitre et al., 2009), abbreviated as SR1-GFP, OSNs that choose this allele for expression coexpress SR1 and tauGFP by virtue of *IRES*-mediated cotranslation from bicistronic messages (Mombaerts et al., 1996). In the four ΔSR1 strains, OSNs that choose the ΔSR1 allele cannot express the SR1 protein from this allele, but instead express either red fluorescent protein (ΔSR1-RFP, Grosmaitre et al., 2009), green fluorescent protein (ΔSR1-GFP), GFP and taulacZ by virtue of an *IRES* (ΔSR1-GFP-lacZ), or the site-specific Cre recombinase (ΔSR1-Cre). The strains were established in a mixed 129 × C57BL/6 background, and are publicly available from The Jackson Laboratory.

Expression patterns in the MOE and the SO

GFP expression in the SR1-GFP strain provides a reference pattern for the four ΔSR1 strains. Labeled OSNs of SR1-GFP mice (SR1-GFP OSNs) reside in a ventral zone of the MOE and in the SO (Fig. 2A1), identical to OSNs expressing the wild-type *SR1* gene. The density of labeled OSNs in the SO of SR1-GFP mice is extraordinarily high (Fig. 2A2). Axons of SR1-GFP OSNs form bundles that project along the septum to the OB, where they coalesce into glomeruli. A single large labeled glomerulus is observed on the lateral surface of the ventral OB of SR1-GFP mice (Fig. 2A3), and a cluster of two to four labeled glomeruli on the medial OB (Fig. 2A4). Dye-tracing studies from the SO have revealed so-called septal glomeruli in the ventromedial OB, which receive axonal projections exclusively from the SO (Lèvai and Strotmann, 2003). The single large labeled glomerulus on the lateral OB of SR1-GFP mice is most likely formed by axons from mOSNs, and some of the glomeruli on the medial OB may receive axons exclusively from sOSNs.

The expression patterns of the three ΔSR1 alleles that encode a marker are also restricted to the ventral MOE and the SO (Fig. 2B). By crossing ΔSR1-RFP with SR1-GFP, we find significantly fewer RFP+ cells than GFP+ cells in 3-week-old mice (Figs. 2C–E): 4,065 ± 618 (average ± SEM, n = 3 mice) RFP+ mOSNs per mouse compared to 11,358 ± 1,264 GFP+ mOSNs per mouse, or 35.4 ± 1.8%. This difference is even more pronounced in the SO: 1,259 ± 60 RFP+ sOSNs per mouse compared to 5,354 ± 274 GFP+ sOSNs per mouse, or 23.5 ± 0.7%. The numbers of labeled sOSNs are similar in ΔSR1-RFP vs ΔSR1-GFP mice, but are reduced by a factor of 4.15 ± 0.35 (average ± SEM, n = 4) in ΔSR1-GFP-lacZ vs ΔSR1-RFP mice.

We discovered extensive biallelic expression in ΔSR1 sOSNs. In SR1-GFP × ΔSR1-RFP compound heterozygous mice, 32.7 ± 3.0% of ΔSR1-RFP sOSNs (n = 3 mice), or 6.6 ± 0.5% of all fluorescently labeled sOSNs (GFP and RFP), are double positive for SR1-GFP, by two-color imaging of intrinsic fluorescence (Figs. 2C–E). In the MOE, only 2.8 ± 0.2 of ΔSR1-RFP cells (n = 3 mice), or 0.74 ± 0.07% of all labeled cells, are double positive for SR1-GFP. We confirmed in heterozygous ΔSR1-GFP-lacZ sOSNs this very high biallelic expression by in situ hybridization (ISH) with probes against *GFP* and *SR1*: 27.7% ± 4.2% of *GFP*+ sOSNs coexpress the wild-type *SR1* allele, and 3.2% ± 0.6% of mOSNs (data not shown). Thus, ΔSR1 sOSNs break the monoallelic expression rule, and in a dramatic way. It remains to be demonstrated that these biallelic expressors transcribe RNA of both *SR1* alleles simultaneously.

Genetic lineage tracing with Cre

Why are there three- to four-fold fewer ΔSR1 OSNs than SR1-GFP OSNs, in contrast to the equal expression frequencies of both alleles of an OR gene with an intact coding region (Feinstein et al., 2004; Mombaerts et al., 1996; Strotmann et al., 2000)? A peculiar difference emerges from the spatial expression patterns of ΔSR1 and wild-type *SR1* alleles within the basal-to-apical dimension of the olfactory epithelium (Fig. 3). We compared the distribution of basal-to-apical positions of the cell bodies of ΔSR1 OSNs with that of OSNs expressing