Molecular and Cellular Neuroscience 46 (2011) 397–408

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

Coordinated coexpression of two vomeronasal receptor V2R genes per neuron in the mouse

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ARTICLE INFO

Article history:
Received 29 August 2010
Revised 18 October 2010
Accepted 7 November 2010
Available online 26 November 2010

Keywords:
Olfaction
Vomeronasal organ
Vomeronasal receptor
Accessory olfactory system
Gene expression

ABSTRACT

The detection of chemosensory stimuli by the sensory neurons of the mouse vomeronasal organ (VNO) is mainly mediated by seven-transmembrane receptors that are encoded by two large gene repertoires, *V1R* and *V2R*. The mouse genome contains 122 intact *V2R* genes, which can be grouped in four families by sequence homology: families A, B, and D (115 genes), and family C (7 genes). Vomeronasal sensory neurons (VSNs) in the basal layer of the VNO epithelium coexpress two *V2R* genes in non-random combinations: one family-ABD *V2R* gene together with one family-C *V2R* gene, such as Vmn2r1 (29% of basal VSNs) or Vmn2r2 (52%). This coordinated coexpression may contribute to the highly specialized sensory response profiles of VSNs, for instance by heterodimerization of a family-ABD with a family-C V2R. The mechanisms that regulate this coordinated cooexpression of two V2R genes per basal VSN are not understood. Among possible models are a sequential and dependent model of expression; a model of random combinations of expression followed by cellular selection of VSNs with appropriate combinations; and a model of direct coordination of gene expression by another gene family such as genes encoding transcription factors. Here, we describe two novel mouse strains with targeted mutations in the family-ABD *V2R* gene *V2rf2* that begin to provide insight into this problem. We observe that the great majority of VSNs that express intact *V2rf2* coexpress Vmn2r1 immunoreactivity, and that the percentage of Vmn2r1 coexpression increases from 3 to 10 wk. Having established this tight coexpression of *V2rf2* with Vmn2r1, we then asked if it is maintained when the coding sequence of *V2rf2* is deleted. We find that the number of VSNs expressing a locus with a targeted deletion in the coding sequence of *V2rf2* that is likely a null mutation, is similar to the number of VSNs that express intact *V2rf2*. But 25% of these VSNs coexpress another family-ABD *V2R*, which is consistent with the absence of negative feedback from the mutated *V2rf2* locus. Interestingly, 9.5% of VSNs expressing the targeted deletion of *V2rf2* now coexpress Vmn2r2. Finally, the marginal region of the VNO epithelium, where immature VSNs are concentrated, has more RNA of family-ABD *V2R* genes than of family-C genes in postnatal wild-type mice. Our results are most consistent with the sequential and dependent model for the coordinated coexpression of two V2R genes per basal VSN.

Introduction

The VNO detects chemosensory stimuli such as pheromones (Tirindelli et al., 2009; Zufall and Leinders-Zufall, 2007). Most VSNs in mouse and rat express a gene of either of two unrelated repertoires that encode G-protein coupled receptors with a putative seven-transmembrane domain structure: vomeronasal receptors *V1R* (Dulac and Axel, 1995; Rodriguez et al., 2002) and *V2R* (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). The cell bodies of $V1R^+$ VSNs reside within the apical layer of the VNO epithelium, and some of these VSNs detect small, volatile molecules (Boschat et al., 2002; Del Punta et al., 2002a; Leinders-Zufall et al., 2000). $V2R^+$ VSNs have their cell bodies in the basal layer, and some respond to nonvolatile ligands such as major histocompatibility (MHC) class I peptides (Leinders-Zufall et al., 2004, 2009), ESP1 (Haga et al., 2010; Kimoto et al., 2005), and major urinary proteins (Chamero et al., 2007; Papes et al., 2010). $V1R^+$ VSNs and $V2R^+$ VSNs can be discerned by the expression of the G-protein subunits Gαi2 and Gαo, respectively. $V1R^+$/Gαi2$^+$/apical VSNs project their axons to the anterior accessory olfactory bulb (aAOB), and $V2R^+$/Gαo$^+$/basal VSNs to the posterior AOB (pAOB) (Berghard and Buck, 1996; Jia and Halpern, 1996).

The deduced amino acid sequence of V2Rs contains a long N-terminal extracellular region (Fig. 1A), which is encoded by five exons and likely forms the ligand-binding site. The mouse has ~280 *V2R* genes; of these, 122 genes have an intact open reading frame and can

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doi:10.1016/j.mcn.2010.11.002

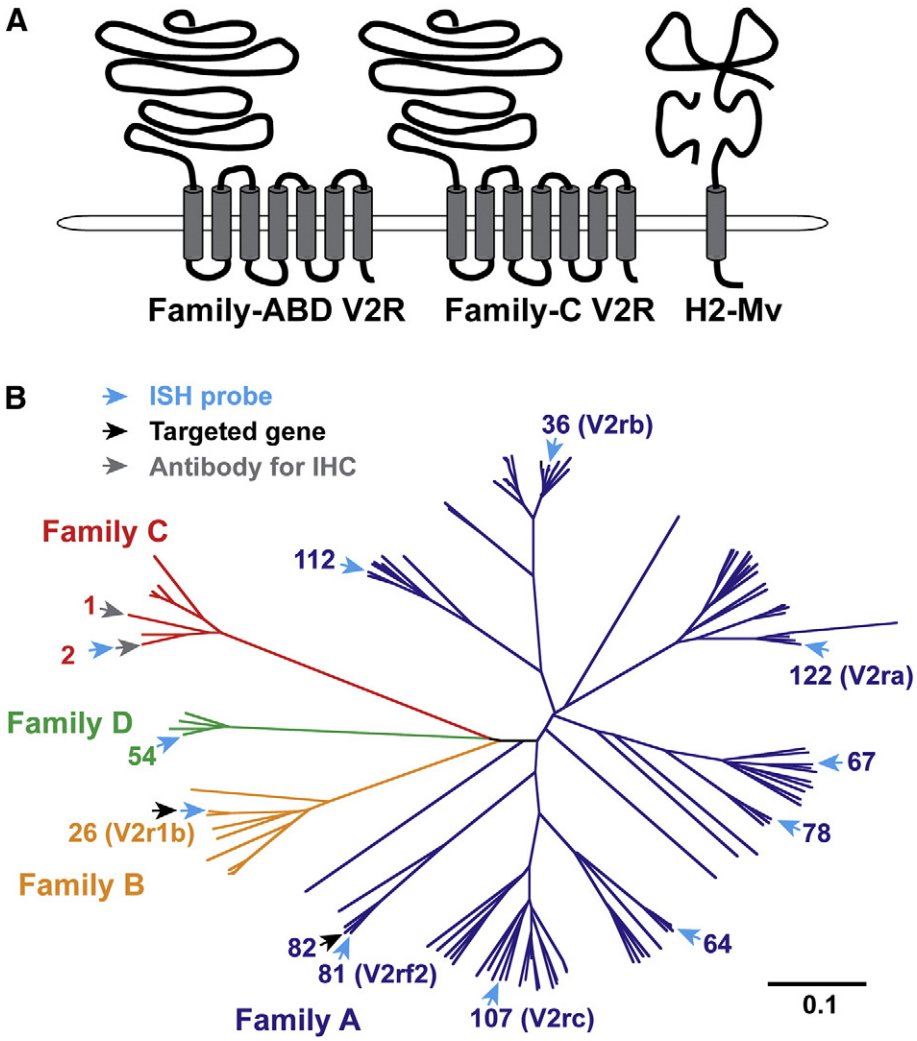

Fig. 1. Protein structures and phylogenetic tree. (A) Schematic structures of family-ABD V2R, family-C V2R, and H2-Mv proteins. A basal VSN coexpresses these three types of proteins in a combinatorial and coordinated fashion. The mechanisms that coordinate these complex patterns of coexpression are not understood. (B) Phylogenetic tree of the 122 intact mouse V2Rs based on deduced amino acid sequences and constructed with njplot. The branches of the four families A, B, C, D are indicated in purple, orange, red, and green, respectively. V2Rs of which coding sequence was used as probes for *in situ* hybridization are marked as blue arrows, with the abbreviated gene number according to the *Vmn2r* nomenclature and the earlier gene name between parentheses. For instance 81 in Family A is an abbreviation for *Vmn2r81*, which is identical to *V2rf2*, the earlier name for this gene. The probe from 82 refers to *Vmn2r82* (= *V2rf1*); this probe is termed *V2rf* (Ishii et al., 2003) and hybridizes to *V2rf1*, *V2rf2*, and *V2rf3*. The two targeted genes are indicated with black arrows, the two V2Rs against which antibodies are directed with red arrows.

be grouped in four families (A, B, D, and C) based on amino acid sequence homology (Fig. 1B) (Shi and Zhang, 2007; Yang et al., 2005; Young and Trask, 2007). There are 115 intact family-ABD genes in mouse. Family-C, which was originally referred to as *V2R2*, contains seven intact genes (Martini et al., 2001; Silvotti et al., 2007).

The consensus of several *in situ* hybridization (ISH) studies in mouse and rat is that family-ABD *V2Rs* are expressed in a mutually exclusive manner, most likely as one gene per neuron. In sharp contrast, ISH probes or antibodies for family-C V2Rs label nearly all basal VSNs in rat and mouse (Martini et al., 2001; Silvotti et al., 2007). Immunoreactivity for family-C members Vmn2r1 and Vmn2r*2* is expressed in a mutually exclusive manner in 29% and 52% of basal mouse VSNs, respectively, and coexpressed with family-ABD *V2Rs* in non-random combinations (Silvotti et al., 2007). In addition, approximately half of basal mouse VSNs express a third multigene family, which encodes non-classical MHC Ib proteins: these genes are termed *H2-Mvs* or *M10s* (Ishii et al., 2003; Ishii and Mombaerts, 2008; Loconto et al., 2003) (Fig. 1A). Coexpression occurs also in non-random combinations among *H2-Mvs*, between *H2-Mvs* and family-ABD *V2Rs*, and between *H2-Mvs* and family-C *V2Rs*.

The emerging picture is thus a complex situation of basal VSNs that coexpress three types of genes in non-random combinations: family-ABD *V2R* (115 genes), family-C *V2R* (seven genes), and *H2-Mv* (nine genes). Such coordinated coexpression of multigene families is not seen in apical VSNs (expressing *V1R* genes) or olfactory sensory neurons (expressing odorant receptor genes), and its mechanisms are not understood. Here, we describe two novel mouse strains with targeted mutations in the *V2R* gene *V2rf2*, which begin to provide insights into the coordinated expression of *V2R* gene families. Together with our observations in wild-type mice, our results fit best with a model in which expression of a family-ABD *V2R* gene determines the particular family-C *V2R* gene that is expressed later.

Results

Targeted mutagenesis of V2R genes

The subjects of this study are two family-ABD *V2R* genes, *V2r1b* from family-B and *V2rf2* from family-A (Fig. 1B). These genes are expressed in the upper/H2-Mv$^-$ and the lower/H2-Mv$^+$ layers of the