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Ric-8B interacts with Gαolf and Gγ13 and co-localizes with Gαolf, Gβ1 and Gγ13 in the cilia of olfactory sensory neurons

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Olfactory sensory neurons are able to detect odorants with high sensitivity and specificity. We have demonstrated that Ric-8B, a guanine nucleotide exchange factor (GEF), interacts with Gαolf and enhances odorant receptor signaling. Here we show that Ric-8B also interacts with Gγ13, a divergent member of the Gγ subunit family which has been implicated in taste signal transduction, and is abundantly expressed in the cilia of olfactory sensory neurons. We show that Gβ1 is the predominant Gβ subunit expressed in the olfactory sensory neurons. Ric-8B and Gβ1, like Gαolf and Gγ13, are enriched in the cilia of olfactory sensory neurons. We also show that Ric-8B interacts with Gαolf in a nucleotide dependent manner, consistent with the role as a GEF. Our results constitute the first example of a GEF protein that interacts with two different olfactory G protein subunits and further implicate Ric-8B as a regulator of odorant signal transduction.

Keywords: Olfactory neurons; Odorant receptors; Ric-8B; Synembryn; Gγ13; Guanine nucleotide exchange factor; G proteins; GEF; Gβ1; Gαolf

Introduction

Odorant signaling must be tightly regulated to allow animals to respond properly to the environmental chemical cues. Olfactory signal transduction is initiated at the cilia of olfactory sensory neurons by the binding of odorants to odorant receptors (ORs), which belong to a large family of G protein coupled receptors (Buck and Axel, 1991). Odorant binding to ORs leads to the activation of the associated heterotrimeric G protein, Golf. Once activated, Gαolf exchanges GDP for GTP, the GTP-bound Gαolf subunit dissociates from the Gβ/γ complex and activates adenylyl cyclase III, leading to increased intracellular levels of cAMP and opening of cyclic nucleotide gated channels. The resulting influx of Na^+ and Ca^{2+} ions ultimately leads to the generation of an action potential in the olfactory neuron axon (Firestein, 2001; Ronnett and Moon, 2002; Mombaerts, 2004).

Even though the major odorant signaling pathway components are already well known, not much is known about how the pathway is regulated. A key point of regulation in G protein-mediated signaling is the inter-conversion between the active GTP-bound and inactive GDP-bound states of the Gα subunit, which can be controlled by regulatory proteins like GTPase Activating Proteins (GAPs) or Guanine nucleotide Exchange Factors (GEFs). GAPs catalyze the formation of the GDP-bound (OFF) state of the Gα subunit, and therefore are considered to work as negative regulators of GPCR signaling. It has been previously shown that some members of the regulators of G protein signaling (RGS) family, which act as GAPs, are expressed in the olfactory sensory neurons, however, to date, no RGS was shown to be directly involved in the regulation of Gαolf (Norlin and Berghard, 2001; Sinnarajah et al., 2001). Actually, many of the known RGS proteins show GAP activity towards Gαi/o or Gαq subunits, but few have been shown to be active towards Gαs like subunits (Willars, 2006).

GEFs have the opposite regulatory effect: they stimulate formation of the GTP-bound (ON) state of the Gα subunit, and therefore are considered to work as positive regulators of GPCR signaling. We have previously described that Ric-8B, a putative GEF that is specifically expressed in olfactory sensory neurons, interacts with Gαolf (Von Dannecker et al., 2005) and amplifies OR signaling through Gαolf in HEK293T cells (Von Dannecker et al., 2006). These results indicate that Ric-8B plays an important role in the regulation of odorant signal transduction in olfactory sensory neurons.

Here we investigated the mechanisms of Ric-8B function. We show that Ric-8B, besides interacting with Gαolf, also interacts with Gγ13. We also show that Gβ1 is the Gβ subunit which is predominantly expressed in the mature olfactory sensory neurons

Abbreviations: GEF, guanine nucleotide exchange factor; GPCRs, G protein coupled receptors; OR, odorant receptor; VNO, vomeronasal organ; Ric-8B, resistance to inhibitors of cholinesterase 8B; RGS, regulator of G protein signaling; GAP, GTPase activating protein; OMP, olfactory marker protein.

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and that the Gβ1 protein is localized to the cilia of olfactory sensory neurons. Pull-down experiments indicate that Gαolf, Gβ1 and Gγ13 are able to form a complex together with Ric-8B. Using antibodies raised against a Ric-8B peptide we show that the Ric-8B protein is enriched in the dendrites and cilia of olfactory sensory neurons. Our results show that Ric-8B assists in trafficking of these G protein subunits to the plasma membrane. Finally, we show that the interaction between Ric-8B and GDP-bound Gαolf is stronger than the interaction between Ric-8B and GTP-bound Gαolf, and that in the presence of Gγ13/Gβ1, Ric-8B remains associated with Gαolf both in the ON and OFF states.

Results

Ric-8B interacts with Gγ subunits

To identify new binding partners for Ric-8B a yeast-two-hybrid olfactory epithelium cDNA library was screened using Ric-8BΔ9 as bait. A group of isolated clones encoded the G protein γ subunits Gγ13 and Gγ8. To determine whether these interactions are specific we used directed yeast-two-hybrid assays to test for Ric-8B and Ric-8BΔ9 interactions with Gγ13, Gγ7 and Gγ8. As shown in Fig. 1, both Ric-8B and Ric-8BΔ9 interact with the three Gγ subunits, but only Ric-8B is able to interact with Gαolf, as previously demonstrated (Von Dannecker et al., 2005). These results indicate that the Ric-8B proteins are able to specifically interact with different Gγ subunits, probably through a conserved structure shared by these proteins. The Gγ7 and Gγ8 subunits are however not expressed in mature olfactory sensory neurons, where Ric-8B and Gαolf are highly expressed. Gγ7 is widely expressed and abundant in the brain, mostly in the striatum, where it was shown to be associated with Gαolf function (Watson et al., 1994; Betty et al., 1998; Schwindiger et al., 2003). Ric-8B and Gαolf are also expressed in the striatum, but at lower levels than in the olfactory epithelium (Belluscio et al., 1998; Von Dannecker et al., 2005). Gγ8 is expressed only in immature olfactory sensory neurons (Ryba and Tirindelli, 1995). The Gγ13 protein, on the other hand, was detected in mature olfactory sensory neurons (Kulaga et al., 2004; Lin et al., 2007) and therefore, is more likely to be the binding partner for Ric-8B in these neurons.

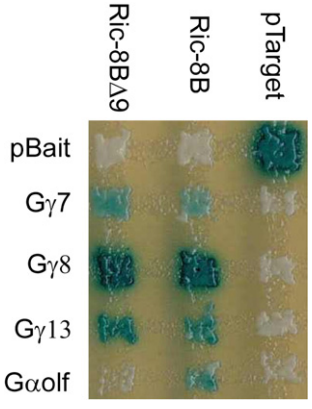

Fig. 1. Ric-8B interacts with Gγ subunits. A. Bait strains expressing full-length Gγ7, Gγ8, Gγ13 and Gαolf were mated with target strains expressing full-length Ric-8BΔ9 and Ric-8B. X-gal was used to score positive interactions. pBait, which constitutively expresses a LexA bait fusion protein that interacts with the fusion protein from pTarget, was used as a positive control.

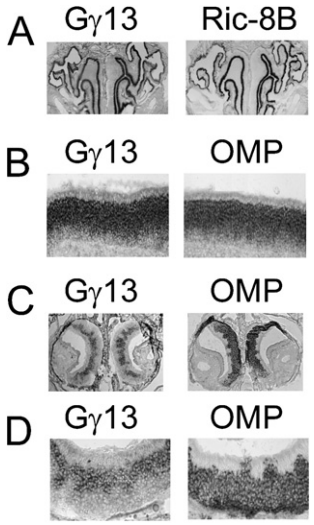

Fig. 2. Expression of Gγ13 in the olfactory system. A. Coronal sections through the olfactory epithelium from P21 mice were hybridized with antisense digoxigenin-labeled probes specific for Gγ13 and Ric-8B. B. Gγ13 and OMP probes hybridize to mature olfactory sensory neurons in the olfactory epithelium. C. Coronal sections through the mouse VNO were hybridized with antisense digoxigenin-labeled probes for Gγ13 or OMP. D. Magnified figures from regions of Fig. 2C. Gγ13 positive neurons are localized to the upper region of the epithelium, while OMP positive neurons are distributed over both the apical and basal regions of the epithelium.

Gγ13 expression in the olfactory system

We next explored the patterns of Gγ13 gene expression in the olfactory epithelium. *In situ* hybridization experiments were performed using digoxigenin-labeled probes and olfactory epithelium tissue sections from P21 mice. A strong hybridization signal for Gγ13 was detected in the olfactory epithelium, comparable to the intensities of signals obtained for Ric-8B (Fig. 2A). Gγ13 is expressed in mature olfactory sensory neurons located in the broad central area of the epithelium, but not in the basal layer that contains immature neurons (Fig. 2B).

We also examined whether Gγ13 is expressed in the vomeronasal organ (VNO). As shown in Fig. 2C, the Gγ13 probe hybridized to neurons in the vomeronasal epithelium. Interestingly, the hybridization signal was detected only in neurons located in the apical region of the epithelium, where Gαi2 and V1R pheromone receptors are normally expressed (Dulac and Axel, 1995; Berghard and Buck, 1996), and not in neurons located in the basal region, where Gαo and V2R pheromone receptors are expressed (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997) (Fig. 2D). The olfactory marker protein (OMP) probe which hybridizes to all mature neurons in both regions of the vomeronasal epithelium, was used as a control (Fig. 2D). It is important to note, however, that unlike Gγ13, Gαolf and Ric-8B are not expressed in the VNO (Berghard and Buck, 1996; Von Dannecker et al., 2005), suggesting that Gγ13 plays a different role in this organ.

Gβ1 is the major G protein β subunit expressed in the olfactory epithelium

Gγ subunits are normally strongly associated with a particular Gβ subunit. We next determined which one of the five known Gβ subunit types (Gβ1–Gβ5) is predominantly expressed in the olfactory