G PROTEIN COUPLED RECEPTORS AND CHEMOSENSATION

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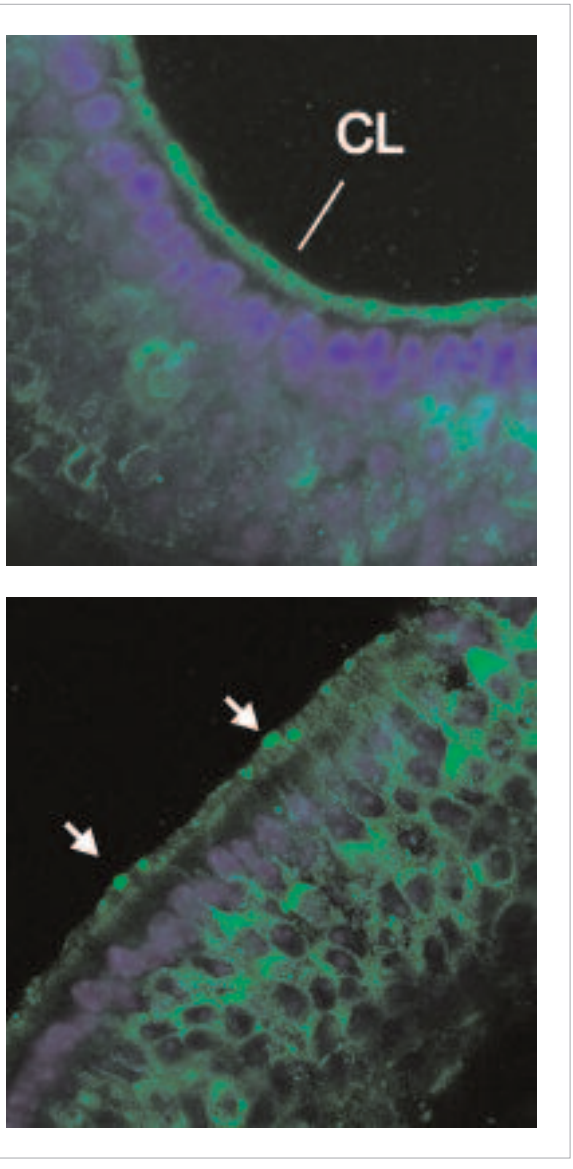

Immunofluorescence showing section through the olfactory epithelium of mouse using the anti-RIC-8B (green) antibody. The RIC-8B protein is located in the ciliar layer of the olfactory epithelium (CL) and also in the dendrites (arrows) of the olfactory nerve cells. Cell nuclei are colored with DAPI (blue)

Animals detect chemical stimuli present in the environment through a large number of receptors which belong to the superfamily of receptors coupled to the G protein (GPCRs). These receptors are expressed in different types of specialized cells, depending on their function. The olfactory receptors are expressed in the olfactory nerve cells, the taste receptors are expressed in the tongue taste buds, and the pheromone receptors, in the nerve cells of the vomeronasal organ. The activation of these receptors, by their ligands, releases a signal which results in the sensorial perception of the various stimuli. In the present project, we intend to analyze the following aspects related to two types of sensorial modality mediated by GPCRs, smell and taste: (1) how the transduction path of the signal of odorants in olfactory nerve cells is regulated *in vivo*. To this end, we intend to verify if the protein RIC-8B, recently identified in our laboratory, plays some role in this regulation. RIC-8B acts as a GEF (Exchange factor of GTP) on GαOLF, the olfactory protein Gα, which is responsible for the signal transduction through the olfactory receptors. (2) Mice present approximately 1000 OR genes, but just one OR gene is expressed in each of the olfactory nerve cells. We intend to investigate the mechanisms that control the regulation of the expression of the OR genes in the olfactory nerve cells. (3) Sweet tastes are detected by a heterodimer of GPCRs, the T1R2 and the T1R3. We intend to isolate molecules that modulate the function of human sweet receptors.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Odorants interact specifically with the olfactory receptors that are present in the cilia of the olfactory nerve cells. This interaction leads to the activation of the G olfactory protein, called GαOLF. We identified a protein called RIC-8B, which is an exchange factor of GTP (GEF) capable of binding to GαOLF and amplifying its activity. In this project we intend to study the functional mechanisms of RIC-8B, as well as determining its role in vivo. To-date, we obtained results which indicate that RIC-8B, in addition to interacting with GαOLF, interacts also with another subunit of heterotrimeric G protein, Gγ13. Our results represent the first example of a GEF which interacts with two different subunits of a heterotrimeric G protein. We also demonstrated that the Gβ1 subunit, is the , subunit predominantly expressed in the olfactory nerve cells, and therefore probable partner of GαOLF and Gγ13. Using an antibody against RIC-8B, we determined that this protein is also found in the cilia of the olfactory nerve cells, together with Gγ13, GαOLF and Gβ1. We also showed that RIC-8B increases the localization of G protein subunits in the plasmatic membrane of the cell. The results obtained up to now suggest two possible functions for RIC-8B in the olfactory nerve cells: RIC-8B may function as an accessory factor, which assists in the assembly and sending of G olfactory protein to the cilia of the olfactory nerve cells, and/or RIC-8B may act as a GEF to amplify the transduction of the signal of odorants. As next step, we will generate knockout mice, using stem cells where the RIC-8B gene is broken. These mice will be evaluated for their olfactory capacity.

The human T1R2/T1R3 receptor expressed in taste buds of the tongue is responsible for the detection of sweet taste and can be activated by different types of sugars, including sweeteners. The receptor has been expressed already in HEK293T cells and is being used through the SELEX method for the selection of aptamers of RNA which are capable of binding specifically to the site of the sucrose receptor. The identification of regulators for this receptor could contribute to the design of sweeteners.

MAIN PUBLICATIONS

Von Dannecker LEC, Mercadante AF, Malnic B. 2005. Ric-8B, an olfactory putative GTP exchange factor, amplifies signal transduction through the olfactory-specific G-protein GαOLF. *J Neurosci.* **25**:3793-3800.

Von Dannecker L, Mercadante A, Malnic B. 2006. Ric-8B promotes functional expression of odorant receptors. *PNAS.* **103**:9310-9314.

Malnic B. 2007. Searching for the ligands of odorant receptors. *Molecular Neurobiology.* **35**:175-181. Review.

Kerr D, Von Dannecker LEC, Davalos M, Michaloski J, Malnic B. 2008. Ric-8B interacts with GαOLF and Gγ13 and co-localizes with GαOLF, Gβ1 and Gγ13 in the cilia of olfactory sensory neurons. *Molecular and Cellular Neuroscience*. In press.

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