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Research Report

A unique transcriptome at the brain–environment interface: Local translation in the rat olfactory epithelium

Marie-Annick Persuy[a,b,*,1,2], Christine Baly[a,b,1], Régine Monnerie[a,b,3], Sylvie Souquere[c,6], Claudia Bevilacqua[d,4], Caroline Dubacq[e,f,5], Gérard Pierron[c,6], Monique Caillol[a,b,1], Jean-Jacques Remy[g,1]

[a]INRA, UR 1197, Neurobiologie de l'Olfaction et Modélisation en Imagerie, F-78350 Jouy-en-Josas, France
[b]IFR 144 Neuro-Sud Paris, France
[c]CNRS, UMR 8122, Institut Gustave Roussy, F-94800 Villejuif, France
[d]INRA, UMR 1313, Génétique Animale et Biologie Intégrative, F-78350 Jouy-en-Josas, France
[e]Université Pierre et Marie Curie-P6, UMR 7102, F-75005 Paris, France
[f]CNRS, UMR 7102, F-75005 Paris, France
[g]INRA, NICN, UMR 6184 CNRS- Université de la Méditerranée, F-13916 Marseille, France

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ABSTRACT

All olfactory epithelium cells, including rapidly self-renewing olfactory sensory neurons (OSN), are continuously subjected to external airborne aggressions. We hypothesized that the apical part of rat olfactory epithelia (AOE) could be the site of a local translation to be able to respond rapidly to external stimuli. We purified significant amounts of mRNAs from AOE. Sequencing of the cDNA library identified 348 mRNA species. Of these, the 220 AOE transcripts encoding proteins with known biological functions were classified in functional groups. The main functional class (40%) coded for defense, detoxification, anti-oxidant stress and innate immunity. Other classes comprised mRNAs encoding functions for neuronal metabolism and life (19%), nuclear transcription control (15%), cell survival and proliferation (13%), RNA processing and translation (12%). They did not contain any known members of the olfactory transduction pathway. The expression of a sub-set of AOE transcripts was investigated in sub-cellular AOE fractions highly enriched in ciliated dendrites and in AOE fractions after forced hemilateral OSN-specific degeneration. All the mRNAs tested were found to be: i) present in enriched ciliated dendrite preparations ii) down-regulated after OSN degeneration iii) co-purified with polysomal fractions, suggesting

* *Corresponding author at:* INRA, UR1197, Neurobiologie de l'Olfaction et Modélisation en Imagerie, 78350 Jouy-en-Josas, France. Fax: +33 134652241.
E-mail addresses: marie-annick.persuy@jouy.inra.fr (M.-A. Persuy), christine.baly@jouy.inra.fr (C. Baly), regine.monnerie@jouy.inra.fr (R. Monnerie), sylvie.souquere@igr.fr (S. Souquere), claudia.bevilacqua@jouy.inra.fr (C. Bevilacqua), caroline.dubacq@snv.jussieu.fr (C. Dubacq), gerard.pierron@igr.fr (G. Pierron), monique.caillol@jouy.inra.fr (M. Caillol), jean-jacques.remy@univmed.fr (J.-J. Remy).

[1] MAP, MC, CB and JJR designed the project and wrote the manuscript.
[2] MAP performed most of the experiments.
[3] RM performed plasmid purification of AOE library.
[4] Cl B conducted laser capture microdissection.
[5] C D helped us for polysomes preparation and helpful criticisms of the manuscript.
[6] GP and SS performed electron microscopy in situ hybridizations.

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their commitment to local translation. We provide strong evidence that the extreme apical side of the olfactory epithelium expresses a unique transcriptome, whose function is not related to olfaction but mainly to defense and survival. The possible local translation of this transcriptome is demonstrated, in supporting cells as well as in olfactory neuron ciliated dendrites.

1. Introduction

The olfactory mucosa (OM) is a complex neuroepithelium composed of various cell types (for a review, see Doty, 2009). Because of its neuroanatomical localization, the OM is directly exposed to environmental attack. The olfactory sensory neurons (OSNs) are the only neurons in direct contact with airborne odorants, pollutants, toxicants and pathogens. A variety of proteins, viruses, drugs or pollutants delivered in the nasal cavity thus have rapid and direct access to the brain via the olfactory route, bypassing the blood–brain barrier (for a review, see Dhuria et al., 2009; Tournier et al., 2009). However, the olfactory mucosa can be considered as constituting a barrier between the brain and the environment (Watelet et al., 2009).

Olfactory sensory neurons have some features that are unique among all neuron types. Mature OSNs are bipolar neurons composed of hair-like ciliated knobs (dendrites) that express the olfactory receptors and the whole odor signaling machinery, cell bodies containing a nucleus and signal-conducting axons. OSN axons enter the brain olfactory bulb, where they connect second-order neurons that lead to other brain areas via the olfactory tract. Individual OSNs are short-lived cells that are replaced throughout life by olfactory precursor cells residing in the olfactory epithelium. Immature neurons thus differentiate, migrate from the base to the surface of the olfactory epithelium and replace aged neurons that have died through a process of programmed cell death. The life-long regeneration of OSN represents one of the few instances of adult neurogenesis. OSNs are bathed in a mucus layer secreted by the Bowman glands and by glia-like sustentacular cells. The latter control the ionic environment of OSNs, they have feet upon basal cells and microvilli extending into the nasal cavity; they regenerate from basal cells, but few data are available concerning their maintenance. Recent data have suggested the existence of a substantial number of different types of cells bearing microvilli at their apex but the function of those microvillous cells is not yet understood (Hegg et al., 2010).

A number of the proteins involved in the metabolic transformation of odorants, the maintenance of homeostasis and detoxification have been localized at the apical part of the olfactory mucosa, in sustentacular and Bowman cells: cytochrome oxidases (Ling et al., 2004; Pataramekin and Meisami, 2005; Piras et al., 2003), heat-shock proteins (Carr et al., 2001; Hegg and Lucero, 2006) and glutathione S-transferases (Whitby-Logan et al., 2004). Proteomic analysis of the upper part of the mucosa has also revealed the presence of such defense proteins in preparations containing mainly olfactory cilia (Mayer et al., 2007, 2009).

As in every epithelium, most cells in the olfactory mucosa are highly polarized. Their ability to express a particular protein in the right place at the right time may be of particular importance in these cells exposed to environmental attack. This may result from the transport of either proteins to their appropriate location or specific mRNAs for local translation. A translational control of localized mRNAs has been well documented during development (St Johnston, 2005) and in highly polarized cells such as neurons, in both dendrites and axons (for a review, see Wang et al., 2010). Importantly, the translation of a localized population of mRNAs in OSN axonal endings in the olfactory bulb has recently been demonstrated (Dubacq et al., 2009).

During the present study, we decided to determine whether the very apical part of the olfactory epithelium that forms a brain–environment interface could be a site for the local translation of a specific mRNA pool. Using immunohistochemistry, electron microscopy and dissection procedures, we demonstrated the presence of mRNAs and the existence of a local translation machinery at the apical part of the olfactory epithelium (AOE). By sequencing a cDNA library made up of AOE, we identified 348 different transcripts (AOEL). But unexpectedly, none of them encoded the proteins known to be involved in the olfactory function of OSN, such as odorant receptors and their odor transduction partners. Rather, this unique transcriptome may support the brain defenses against environmental hazards.

2. Results

2.1. *The apical part of the olfactory mucosa contains RNAs*

First of all, we determined the presence of RNAs in the AOE cellular compartment using two different protocols for physical tissue dissections. Laser capture microdissection (Fig. 1A) was able to capture discrete patches of the most apical cytoplasm of epithelial cells. In parallel, we developed another dissection technique using monitored micromanipulators and an inverted microscope, designed to physically disrupt and capture the upper part of the olfactory mucosa (Fig. 1B). It was thus possible to isolate fragments that were about 5 μm thick and 50 to 75 μm long.

Both methods – the former already validated for RNA isolation (Bevilacqua et al., 2010) and the latter developed in our laboratory – enabled the purification of RNAs from samples of the upper part of the epithelium, but the yields remained low (a few ng per sample). Among these RNAs, we looked for the presence of a very abundant species (28S ribosomal RNA) that it was possible to detect after RT-PCR amplification (Fig. 1C). However, the low RNAs yield and the