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Yeast That Smell

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Abstract

The fundamental mechanism of olfactory receptor activation has been conserved from yeast to humans. Engineered yeast cells can smell some of the same odorants as humans can, which makes yeast an ideal model system for studying human olfaction. Furthermore, if engineered yeast cells are incorporated into sensory arrays, they can be used as biosensors or artificial noses.

Keywords: Yeast, olfactory receptor, G protein-coupled receptor, biosensor, smell

The olfactome

The sense of smell is one of the most complex and elaborate physiological systems in humans and other animals. Based on genome sequences analysis, humans have about 350 olfactory receptors (ORs) and mice have about 1000 ORs (Malnic 2007). Nonetheless, humans can sense as many as 10,000 to 100,000 chemicals as distinct odors. This discrepancy between number of receptors and number of odors perceived suggests that the mammalian olfactory system uses a combinatorial code, such that each OR recognizes multiple odorants and most odorants are recognized by several ORs (Malnic et al. 1999). The limited pharmacological studies conducted to date on ORs support this hypothesis.

Perception of odorants starts with the stimulation of ORs on sensory neurons within the nasal olfactory epithelium, resulting in the activation of an adenylyl cyclase (AC), and subsequent opening of cyclic nucleotide-activated, nonselective cation channels (Fig. 1A) (Mombaerts 2004). Electrical signals generated from OR activation are propagated in turn to the main olfactory bulb, the olfactory cortex, higher cortical areas and limbic structures of the brain, where eventually a perception of odor is formed (Reed 2003).

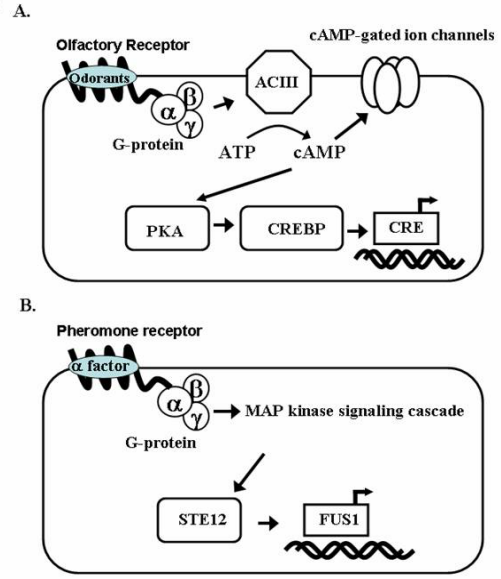

Figure 1: Conservation of OR-activated signal transduction pathways between yeast and mammals. (a) Schematic of the mammalian olfactory signal transduction pathway in the olfactory epithelium. Binding of odorants to olfactory receptor activates heterotrimetric G protein, which subsequently results in activation of the AC activity, elevated cAMP level, and opening of cAMP-gated ion channels. Synthesis of cAMP activates PKA and in turn upregulates many genes. OR, olfactory receptor; ACIII, adenylyl cyclase; PKA, protein kinase A; α, β, γ, heterotrimeric G protein α subunit, β subunit, γ subunit; CRE, cAMP responsive element. (b) Schematic of the yeast mitogen-activated protein kinase signal transduction pathway. Activation of pheromone receptor results in activation of heterotrimetric G protein, stimulation of MAP kinase signaling cascade and in turn upregulation of many genes.

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OR genes are members of the G protein coupled receptor (GPCR) superfamily, which are cell surface proteins with a conserved structure of seven transmembrane domains (Buck and Axel 1991). Understanding OR activation is central to decoding human olfaction; the combinatorial map of OR-odorant pairs that determine the perception of distinct odors is far from complete. Given the difficulty of in situ pharmacology in the olfactory epithelium, a practical approach to obtaining a comprehensive OR-odorant coding map would be to determine the interaction of odorants and ORs in a simple heterologous cell system. Because the basic molecular mechanism of OR activation is conserved evolutionarily from yeast to humans, yeast has attracted attention as a potential platform for 'deorphanizing' the human olfactome.

Pheromone sensing in yeast

Haploid yeast cells exist in two cell types — *a* or α cells. *a* and α cells can mate with each other to form diploids. Haploid yeast cells initiate mating with the opposite cell type upon "smelling" the pheromone produced by mating partners. The protein that "smells" the pheromone released from the opposite cell type is a cell type-specific GPCR, Ste2 in *a* cells or Ste3 in α cells. Stimulation of the yeast GPCR by pheromone leads to activation of a mitogen-activated protein (MAP) kinase signal transduction pathway and the induction of several genes, including *FUS1*, a gene involved in cell fusion (Fig. 1B) (King et al. 1990; Dohlman 2002).

Many mammalian GPCRs have been functionally expressed in the yeast *Saccharomyces cerevisiae*. Stimulation of such heterologous GPCRs by ligands leads to activation of the MAP-kinase pathway in yeast, which can be monitored in colorimetric, fluorometric or growth assays using appropriate transcriptional reporters fused to the *FUS1* promoter (Klein, Paul et al. 1998). The significant conservation of GPCR-activation-machinery between yeast and mammalian cells, the null background of mammalian GPCRs in the yeast system, the easy manipulation of the yeast genome genetically and molecularly, and the robust growth of yeast cells, all render yeast an excellent platform for pharmacological studies of mammalian GPCRs (Silverman et al. 1998).

Olfactant sensing in yeast

Study OR-activation in yeast, an analogous approach was taken as that of studying the GPC activation in yeast: Two key mammalian olfactory components, an OR and the G protein α subunit, $G_{\alpha olf}$, expressed in the olfactory epithelium, were engineered into a yeast strain to replace yeast GPCRs *STE2* and *STE3* genes and the yeast G_α gene, *GPA1*. GPCRs couple to downstream effects through a heterotrimeric complex consisting of three subunits-G_α, G_β and G_γ. G_α mediates the interaction between the receptor and the $G_{\beta\gamma}$ heterodimer. To maximize G_α subunit interaction with heterologously expressed mammalian ORs while maintaining its association with yeast $G_{\beta\gamma}$ heterodimer, a sandwich G_α protein was constructed by replacing the receptor-coupled domain of yeast Gpa1 with the corresponding rat $G_{\alpha olf}$ (Fig. 2A). This sandwich G_α gene was used to replace the genomic copy of *GPA1* to create yeast strain Y3623*. Since amino acid sequences of $G_{\alpha olf}$ show high similarity to that of $G_{\alpha s}$, which is also a G_α subunit that activates AC in mammals, the functional integrity of the engineered Y3623 yeast strain can be examined by determining whether the $G_{\alpha s}$–coupled human adenosine receptor 2B (A_{2B}) can couple to $G_{\alpha olf}$ and be activated by its specific ligand 5'-N-ethylcarboxamidoadenosine (NECA) (Mirabet et al. 1999). The human A2B receptor expressed in Y3623 showed 50-100 fold dose-dependent response to NECA (Sigma-Aldrich), but not to non-specific ligands, as determined by measuring the β-galactosidase activity with expression of the P_{FUS1}-*LacZ* reporter gene on a vector (Fig. 2B). Thus, a yeast-based assay was developed to study the olfactory receptors. Rat OR I7 or human OR 17-40 expressed under the control of a strong yeast promoter PGK1 in a yeast *LEU2*-marked 2μ vector was transformed into Y3623, respectively. The functional activation of rat OR I7 or human OR 17-40 by its reported odorants was examined. Although we were only able to detect moderate (~ 2 fold) activation sporadically and could not observe the dose-dependent response consistently, Minic *et al.* reported agonist-dependent activity in a similarly engineered yeast system (Minic et al. 2005). These authors found that both rat OR I7 and human OR 17-40 functionally respond to odorants as they do in mammalian cells. The response of ORs to their ligands in yeast was enhanced when the receptors were induced to a high level at 15°C. Since the concentrations of the odorant responses by the ORs

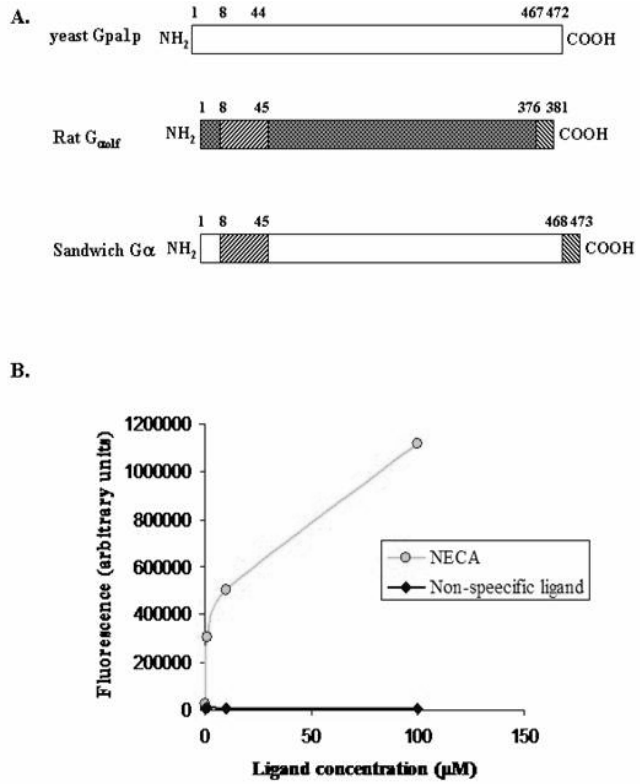

Figure 2: Engineering of the olfactory yeast strain Y3623. (a) Schematic illustration of sandwich Gα subunit in Y3623. Amino acids (aa) SKTAEDQGVDEKERREANKKIEKQLQKERLAYKATHR from 8 to 44 and the last 5 aa QYELL of rat $G_{\alpha olf}$ replaced corresponding *GPA1* domain. This was done by fusion PCR. PCR primers used to amplify rat $G_{\alpha olf}$ (a gift from Dr. Randall Reed) and yeast *GPA1* are available upon request. Open rectangle represents the aa sequences of Gpa1p and shaded rectangle represents the aa sequences of rat $G_{\alpha olf}$. (b) Response of human A_{2B} receptor expressing yeast strain to NECA, but not non-specific ligands. Human A_{2B} receptor and a vector containing the *FUS1p-LacZ* reporter gene were transformed into the olfactory yeast strain Y3623. The resulting yeast cells were grown overnight to exponential phase, seeded to a 96-well plate in 100 μl, and treated with NECA at increasing concentrations for 4 hours at 30°C. β-galactosidase activity was determined by addition of 0.5 mM fluorescein di-β-D-galactopyranoside (FDG, Molecular Probes). Fluorescence was measured in a Wallac Victor microplate reader with 485-nm excitation and 535-nm emission.

concentrations of the odorant responses by the ORs lie in a very narrow range in Minic et al.'s paper, we speculate that the range of ligand concentration could be critical for observing activation of the OR, or that the engineered yeast strain background influences the specificity of the odorants. Using the engineered olfactory yeast cells made by Minic et al., Marrakchi et al. developed an olfactory biosensor (Marrakchi et al. 2007). Yeast cells expressing OR 17-40 immobilized onto interdigitated thin film microelectrodes specifically recognize the ligand for OR 17-40. Thus, yeast cells could potentially be used as artificial noses.

Recently, Radhika et al. developed "olfactory yeast" by engineering a multistep mammalian olfactory signaling pathway into the yeast *Saccharomyces cerevisiae* (Radhika et al. 2007). In the mammalian olfactory system, stimulation of ORs by odorants activates the $G_{\alpha olf}$ subunit, which interacts with type III adenylyl cyclase (ACIII) and leads to synthesis of cyclic AMP (cAMP). Besides opening cyclic nucleotide gated channels, cAMP can activates CREBP, a DNA binding factor that activates transcription via cAMP-response elements (CREs) (Fig. 1A). To establish an olfactory signal transduction pathway independent of any yeast signal transduction pathways, Radhika et al. cloned all the main components of the mammalian cAMP reponse pathway into yeast — ACIII and CREBP — along with mammalian ORs and three mammalian subunits of the olfactory heterotrimeric G protein (rat $G_{\alpha olf}$, $G_{\beta 2}$ and $G_{\gamma 5}$),. In addition, a GFP reporter gene expressed under the control of human CRE elements was integrated into the yeast genome so that activation of the olfactory signaling pathway in yeast could be monitored by the expression of GFP. In this olfactory yeast, activation of the OR by odorants only functions through the artificial olfactory signaling pathway. Functional expression of rat OR I7 and response to its odorants were observed. Interestingly, Radhika et al. identified a receptor that responds to 2, 4-dinitrotoluene, a mimic for the explosive trinitrotoluene (TNT), through screening a library of "orphan" receptors. This demonstrates that olfactory yeast could be adapted to high-throughput screens to identify ligands for orphan ORs and it suggests that the olfactory yeast could be used as biosensors for environmental monitoring.

Perspective

With recombinant DNA and genetic engineering technologies, olfactory yeast strains have been produced that can "smell" some of the same chemicals that humans do. As additional receptors are expressed and profiled in these strains, it will become possible to address the question of how the combinatorial olfactory mechanism allows such a large ensemble of receptors to function seamlessly together. These strains will provide invaluable clues as to the encoding and decoding mechanisms required for discrimination of distinctive odors, along with perception of spatial and temporal cues. Yeast cells have a long life-time and have the characteristics of low-cost preparation and robust growth, which makes olfactory yeast attractive candidates for development as biosensors or an artificial nose. The problems with the current generation of olfactory yeast are their sensitivity, specificity and versatility. However, successful construction of mammalian olfactory signaling pathway in yeast portends that fine-tuning the pathway for yeast that smell better is possible. To this end, it is anticipated that in the future having a yeast-based biosensor or yeast nose in daily life will not be science fiction.

*Y3623 is made from a yeast strain derivative of Cy1141 (Klein et al. 1998).

The genotype of Y3623 is: *Matα FUS1p-HIS3 gpa1Δ::gpa1(7)-$g_{\alpha olf}$(8-48)-gpa1- $g_{\alpha olf}$(5) ste18g6-3841 ste3Δ1156 far1Δ1442 sst2Δ2 tbt1-1 ste14::trp1::LYS2 his3 leu2 lys2 trp1 ura3 can1*

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