Молекулярные и клеточные механизмы обоняния и вкуса

Колесников С.С.

Институт биофизики клетки РАН
Introduction in Cell Physiology.
### Bilayer permeability for different species

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
<th>Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gases</strong></td>
<td>CO$_2$, N$_2$, O$_2$</td>
<td>Permeable</td>
</tr>
<tr>
<td><strong>Small uncharged polar molecules</strong></td>
<td>Ethanol, H$_2$O, Urea</td>
<td>Permeable</td>
</tr>
<tr>
<td><strong>Large uncharged polar molecules</strong></td>
<td>Glucose, fructose</td>
<td>Impermeable</td>
</tr>
<tr>
<td><strong>Ions</strong></td>
<td>K$^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$, HCO$_3^-$, HPO$_4^{2-}$</td>
<td>Impermeable</td>
</tr>
<tr>
<td><strong>Charged polar molecules</strong></td>
<td>Amino acids, ATP, glucose 6-phosphate, proteins, nucleic acids</td>
<td>Impermeable</td>
</tr>
</tbody>
</table>
Mosaic structure of the plasma membrane
Typical intracellular and extracellular ion concentrations

<table>
<thead>
<tr>
<th>ION</th>
<th>CELL (mM)</th>
<th>BLOOD (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAMMALIAN CELL (VERTEBRATE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>139</td>
<td>4</td>
</tr>
<tr>
<td>Na⁺</td>
<td>12</td>
<td>145</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>X⁻</td>
<td>138</td>
<td>9</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>&lt; 0.0002</td>
<td>1.8</td>
</tr>
</tbody>
</table>
**Selectivity**: Na⁺, K⁺, H⁺, Ca²⁺, Cl⁻, cationic, non-selective

**Gating**: voltage, ligand binding, pH, mechanical stimuli, temperature, light
Cell surface receptors

G-protein coupled receptors

Ionotropic receptors

Receptor tyrosine kinase

Receptor histidine kinase

Tyrosine kinase - linked receptors

Receptor tyrosine phosphatase

Receptor serine/threonine kinase

Receptor guanylate cyclase
G-protein coupled receptors

Seven transmembrane-spanning (7TM) receptors
Heptahelical receptors
Serpentine receptors

peptides, proteins

GPCRs > 1000

16 α-subunits
5 β-subunits
14 γ-subunits

Adenylyl cyclase
Phospholipases Cβ and A2
cGMP phosphodiesterase
Ca^{2+} channels
K channels
PI3 kinase
Receptor guanylyl cyclase

Receptor domain

GC domain

GTP $\rightarrow$ cGMP
Cyclic nucleotides

3’,5’-cyclic AMP (cAMP)

3’,5’-cyclic GMP (cGMP)

AC
ATP → cAMP → AMP

PDE

GC
GTP → cGMP → GMP

PDE
1,2-Diacylglycerol (DAG) is converted to Inositol 1,4,5-trisphosphate (IP₃) by Phospholipase C. PI 4,5-bisphosphate (PIP₂) is a precursor in this process.
cAMP signaling

Stimulatory hormone \{ Epinephrine, Glucagon, ACTH \}

Exterior

Cytoplasm

Receptor for stimulatory hormone

Activation of E

Stimulatory G protein complex

G_{\beta\gamma} G_{\alpha S}

Adenylyl cyclase (E)

cAMP

Inhibitory hormone \{ PGE_1, Adenosine \}

Inhibition of E

Inhibitory G protein complex

G_{\alpha i} G_{\beta\gamma}

Receptor for inhibitory hormone

EPAC      PKA      Ion channels
PLC signaling
Olfaction
Complexity of the mammalian olfactory system

Grueneberg ganglion

VNO

Septal organ

MOE

MOB

AOB

Brain
Main olfactory epithelium

(A) Olfactory sensory neuron
(B) Supporting cell
Population of chemosensory cells in MOE

Odorant receptors

Trace amine receptors

Receptor-GC

Ciliary OMP-positive

Microvillar OMP-negative

Population of chemosensory cells in MOE

Odorant receptors

Trace amine receptors

Receptor-GC

Ciliary OMP-positive

Microvillar OMP-negative
Physiological era
Distinct cell subpopulations contribute additively to electroolfactogram

\[ V_t = \sum a_i N_i \Psi_i \]
Responses of the main olfactory epithelium to different odorants

1 сек

1 мВ

евгенол, 28 мкМ

анизол, 29 мкМ

цинеол, 29,5 мкМ

изомасляная к-та, 26 мкМ

изовалериановая к-та, 25 мкМ

2-гептанон, 29 мкМ
cAMP generation in olfactory cilia

Boekhoff, Breer, PNAS 1992
Patch clamp method for recording channel activity
Electrical excitation of olfactory neurons by odorants

- [Graph showing the time course of electrical excitation with lilial as the odorant, and the peak current recorded over time.]
- [Graph showing the relationship between Lilial concentration (µM) and peak current (pA).]
cAMP-gated current (pA) vs. cAMP, µM

-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6

0 0.1 0.5 2 5 50
AC transduction cascade in olfactory cilia

- Receptor
- Odorant
- Ca2+
- Cl-
- Na+
- PDE1
- ACIII
- cAMP-gated channel
- CNGA2
- Arrestin
- RK
- Golf
- CaM
- cAMP
- Cl-in ~ 40-100 mM
- Cl-out ~ 55-93 mM
Adaptation
Definition of cellular adaptation

A

B

C

Control response

Adapted response

Stimulation

Stimulus intensity

Normalized response
Two pulse protocol for assaying adaptation
Adaptation of olfactory sensory neurons is mediated by Ca$^{2+}$

A

3 mM Ca$^{2+}$

Cineol, 300 $\mu$M

1 sec

50 pA

B

3 mM Mg$^{2+}$, no Ca$^{2+}$

Cineol, 300 $\mu$M

Kurahashi & Shibuya, Brain Res., 1990
Ca2+-dependent elements of the olfactory transduction cascade
Olfactory CNG channel

After Trudeau & Zagotta, J.Biol.Chem. 2003
**Normalized open probability**

- **Control**: $K_{1/2} = 1.4 \, \mu\text{M}$
- **Calmodulin**: $K_{1/2} = 29 \, \mu\text{M}$

**Diagram Details**
- X-axis: cAMP concentration, \( \mu\text{M} \)
- Y-axis: Normalized open probability

**Graph Elements**
- Two curves: one for Control and one for Calmodulin
- The Control curve is red, and the Calmodulin curve is blue.
Molecular era
The Nobel Prize in Physiology or Medicine 2004 for the discovery of odorant receptors

Richard Axel

Linda Buck
<table>
<thead>
<tr>
<th>Species</th>
<th>Number of OR genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>388</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>399</td>
</tr>
<tr>
<td>Macaque</td>
<td>326</td>
</tr>
<tr>
<td>Mouse</td>
<td>1063</td>
</tr>
<tr>
<td>Rat</td>
<td>1259</td>
</tr>
<tr>
<td>Dog</td>
<td>822</td>
</tr>
<tr>
<td>Caw</td>
<td>1152</td>
</tr>
<tr>
<td>Opossum</td>
<td>1198</td>
</tr>
<tr>
<td>Chicken</td>
<td>300</td>
</tr>
<tr>
<td>Xenopus</td>
<td>1024</td>
</tr>
<tr>
<td>Zebra fish</td>
<td>155</td>
</tr>
<tr>
<td>Fugu</td>
<td>86</td>
</tr>
<tr>
<td>Drosophila</td>
<td>62</td>
</tr>
<tr>
<td>C.elegans</td>
<td>1278</td>
</tr>
</tbody>
</table>

Class I (fish-like) ~ 10%
Class II ~ 90%
# Receptors operative in MOE

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligands</th>
<th>Origin</th>
<th>Proposed functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>odorant GPCRs N = 350-1300</td>
<td>general odors</td>
<td>food environment</td>
<td>odor recognition, discrimination, attraction, repulsion</td>
</tr>
<tr>
<td></td>
<td>MHC class I peptides</td>
<td>urine body secretions</td>
<td>social recognition of other strains</td>
</tr>
<tr>
<td>trace amine GPCR N = 3-20</td>
<td>volatile amines</td>
<td>urine</td>
<td>stress response gender recognition</td>
</tr>
<tr>
<td>GC-D N = 1</td>
<td>CO</td>
<td>atmosphere</td>
<td>avoidance behavior</td>
</tr>
<tr>
<td></td>
<td>(\text{CO}_2)</td>
<td>urine</td>
<td>Detection of cues related to Hunger, satiety, or thirst</td>
</tr>
<tr>
<td></td>
<td>uroguanyline</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>guanylin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
GC-cGMP transduction cascade in olfactory cilia

Guanylyl cyclase-D

PDE2

cGMP-gated channel

CNGA3

cGMP

ΔF/F=10%

0.04% CO₂

CO₂+Diltiazem

CO₂+wash

Leinders-Zufall et al., PNAS 2007

Hu et al., Science 2007

uroguanylin

guanylin
Encoding
Tree types of cone photoreceptors
Axons of M71-expressing OSN converge into two glomeruli

M71-LacZ transgenic mouse

Vassalli et al., Cell, 2002
Glomeruli

Olfactory sensory neurons

OR1

OR2

OR3

Olfactory bulb

Glomeruli
OSN responses to different odorants

Odorant coding

- Odorant 1
  - ORs
  - Code: [ Gray, Orange, Cyan, Red, Gray ]

- Odorant 2
  - ORs
  - Code: [ Gray, Gray, Blue, Red, Blue ]

- Mixture
  - ORs
  - Code: [ Gray, Orange, Cyan, Red, Gray, Blue, Gray, Blue, Red, Blue ]
Ca2+ imaging assay

Light source

Dichroic

objective

cell

Fura-2

Fluo-4

CCCD camera
Imaging of rat olfactory bulb loaded with the voltage-sensitive dye JPW3028

Johnson and Leon, J. Comp. Neurol. 2000
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Effect</th>
<th>Olfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Amino-s-triazole</td>
<td>Mouse</td>
<td>Attracts females</td>
<td>?</td>
</tr>
<tr>
<td>2,5-Dimethylpyrazine</td>
<td>Mouse</td>
<td>Delays puberty in females</td>
<td>VNO</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>Mouse</td>
<td>Extends estrus</td>
<td>VNO</td>
</tr>
<tr>
<td>4-Ethylphenol</td>
<td>Mouse</td>
<td>Attracts females and repels males</td>
<td>VNO</td>
</tr>
<tr>
<td>2-sec-Butyl-4,5-dihydrothiazole</td>
<td>Mouse</td>
<td>Attracts females and repels males; induces</td>
<td>VNO</td>
</tr>
<tr>
<td>2,3-Dehydro-exobrevicomin</td>
<td>Mouse</td>
<td>Attracts females and repels males; induces</td>
<td>VNO</td>
</tr>
<tr>
<td>2-Methylbut-2-enal</td>
<td>Rabbit</td>
<td>Attracts pups to nipples</td>
<td>MOE</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>Hamster</td>
<td>Induces copulation in males</td>
<td>VNO</td>
</tr>
<tr>
<td>(Z)-7-Dodecenyl acetate</td>
<td>Asian elephant</td>
<td>Attracts males</td>
<td>?</td>
</tr>
<tr>
<td>5-a-Androst-16-en-3-one</td>
<td>Pig</td>
<td>Facilitates mating in females</td>
<td>MOE</td>
</tr>
<tr>
<td>Sodefrin</td>
<td>Newt</td>
<td>Attracts females</td>
<td>VNO</td>
</tr>
</tbody>
</table>
### Number of genes (pseudogenes)

<table>
<thead>
<tr>
<th>Species</th>
<th>V1R</th>
<th>V2R</th>
<th>TRPC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>5 (115)</td>
<td>0 (20)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>0 (116)</td>
<td>0 (17)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>Macaque</td>
<td>?</td>
<td>0 (11)</td>
<td>?</td>
</tr>
<tr>
<td>Mouse</td>
<td>187 (121)</td>
<td>121 (158)</td>
<td>1</td>
</tr>
<tr>
<td>Rat</td>
<td>106 (66)</td>
<td>79 (142)</td>
<td>1</td>
</tr>
<tr>
<td>Dog</td>
<td>8 (33)</td>
<td>0 (9)</td>
<td>1</td>
</tr>
<tr>
<td>Caw</td>
<td>40 (45)</td>
<td>0 (16)</td>
<td>?</td>
</tr>
<tr>
<td>Opossum</td>
<td>98 (30)</td>
<td>86 (79)</td>
<td>?</td>
</tr>
</tbody>
</table>
Key signaling molecules identified in VNO neurons

- **Apical neurons:** V1R, Gi2, TRPC2
- **Basal neurons:** V2R, Go, TRPC2
Transduction cascade in VNO neurons

V1R/V2R Pheromone

αβγ

PIP$_2$

IP$_3$

DAG

TRPC2

Ca$^{2+}$

AA-gated channel

G$_{i2}/G_0$

AA

CaM

PLC

DAG lipase

IP$_3$
Taste

Sweet
Bitter
Sour
Salty
Umami
Fat?
Distribution of taste buds in the rodent oral cavity

- Soft palate
- Epiglottis
- Fungiform
- CV
- Foliate
Taste bud

Lindemann, Nature 2001
Detection of ionic stimuli with ion channels

\[ J = 10^5 \text{ Na}^+ \text{ ions/s} \]

\[ \Delta Q = C \Delta V \sim 10^{-11} \text{F} \times 10^{-3} \text{V} = 10^{-14} \text{C} \sim 10^5 \text{ e} \]

1 pA \sim 10^7 \text{ e/sec}
amiloride-sensitive Na⁺ channels

H⁺-sensitive cation channels
Molecular era. Gustducin

McLaughlin et al. Nature 1992
Taste behavior of gustducin-null mice

\[ R = \frac{\delta V_1}{\delta V_1 + \delta V_2} \]

- **Preference**: \( R > 0.5 \)
- **Indifference**: \( R = 0.5 \)
- **Aversion**: \( R < 0.5 \)

- • - gustducin KO
- o - wild type

cGMP
rhodopsin
transducin
cGMP-gated channel
Rh-kinase
arrestin
guanylyl cyclase
αβγ
PDE
* phosphodiesterase
phosphorylated rhodopsin
arrestin
Rh-kinase
guanylyl cyclase
GC
 Phototransduction cascade
Taste-specific G-protein coupled receptors

I. T1R family:
   T1R1, T1R2, T1R3

II. T2R family:
   T1R1 – T2R37

Hoon et al., Cell 1999

Adler et al. Cell 2000
# Taste receptor repertoire

<table>
<thead>
<tr>
<th>Species</th>
<th>T1R</th>
<th>T2R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Macaque</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>Dog</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Caw</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Opossum</td>
<td>3</td>
<td>29</td>
</tr>
</tbody>
</table>
Ca2+ imaging assay

- Light source
- Dichroic
- Objective
- Cell
- Fura-2
- Fluo-4
Responses of HEK-293 cells co-expressing T1R2 and T1R3 to sweet compounds.
Responses of HEK-293 cells transfected with T2R to bitter compounds

Adler et al.
Cell 2000
Taste bud innervation
### Taste nerve responses in T1R KO mice

<table>
<thead>
<tr>
<th></th>
<th>Umami</th>
<th>Sweet</th>
<th>Bitter</th>
<th>Sour</th>
<th>Salty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
</tr>
<tr>
<td>$T1r1$-KO</td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
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<tr>
<td>$T1r2$-KO</td>
<td><img src="#" alt="Response" /></td>
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<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
</tr>
<tr>
<td>$T1r3$-KO</td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
</tr>
<tr>
<td>$T2r5$-KO</td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
<td><img src="#" alt="Response" /></td>
</tr>
</tbody>
</table>

Chandrashekar et al., *Nature* 2006
Family of taste G-protein coupled receptors

I. T1R family:

T1R1, T1R2, T1R3

II. T2R family:

T1R1 – T2R36
Knock-out of PLC$\beta$2 and TRPM5 diminishes sweet, bitter, and umami responsiveness.

Chandrashekar et al., Nature 2006
Taste bud cells

Type I

Type II

Type III

Basal cells

Afferent nerve
Sapid molecules

Taste receptor

G-protein

PLCβ2

TRPM5

Ca2+ store

Ca2+

Type II cell
How do type II cells signal to the taste nerve?

Type I: salty
Type II: sweet, bitter, umami
Type III: sour

T1R/T2R receptors
Gustducin
Phospholipase Cβ2
TRPM5
P2X2/P2X3-null mice exhibit the complete loss of behavior and neuronal responses to stimuli of all taste modalities.

Chorda tympani nerve responses

Finger et al., Science 2005
Depolarization of a taste cell results in mobilization of intracellular Ca\textsuperscript{2+} in adjacent COS-1 cells.
Classical exocytotic synapse

Engelman, MacDermott, Nat.Rev.Neurosci 2004
ATP secretion is independent of intracellular Ca\(^{2+}\)
The non-canonical synaptic transmission in the taste bud

- Taste receptor
- TRPM5
- G-protein
- Phospholipase Cβ2
- ATP-permeable channels
- Sodium (Na+) channels
- IP3
- DAG
- Ca2+
- ATP
- Nerve ending
- VG Na+ channel
- P2X
- Sapid molecules
- Ca2+ store

Romanov et al., EMBO J., 2007
Taste bud cells

Type III
- Sour
- PDK2L1 channel
- VG Ca\(^{2+}\) channels
- Exocytosis

Type II
- Bitter, sweet umami
- G-protein
- Connexin hemichannel
- Classical synapse

Type I
- Salty
- ENa channels
- Na\(^{+}\)
- TRPM5
- Ca\(^{2+}\)
- ADP
- ATP
- P2X
- Afferent nerve ending
- AP
- Ca\(^{2+}\)-gated Cl\(^{-}\) channel
- Ecto-nucleotidase
Taste isn’t just for taste buds anymore

Finger, Kinnamon
F1000 Biology Reports 2011
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell type</th>
<th>Signaling proteins</th>
<th>Chemical stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory epithelium of the nose</td>
<td>solitary chemosensory cells</td>
<td>T2Rs, α-gustducin, PLCβ2, TRPM5</td>
<td>denatonium</td>
</tr>
<tr>
<td>Main olfactory epithelium</td>
<td>olfactory sensory neurons</td>
<td>TRPM5</td>
<td>2-heptanone</td>
</tr>
<tr>
<td></td>
<td>microvillar cells</td>
<td>PLCβ2, TRPM5</td>
<td></td>
</tr>
<tr>
<td>Non-sensory epithelium of VNO</td>
<td>solitary brush-like cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>?</td>
</tr>
<tr>
<td>Tracheal epithelium</td>
<td>solitary brush cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>?</td>
</tr>
<tr>
<td>Alveolar epithelium,</td>
<td>solitary brush cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>?</td>
</tr>
<tr>
<td>Gastrointestinal tract epithelia:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stomach</td>
<td>solitary brush cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>bitter?</td>
</tr>
<tr>
<td>colon</td>
<td>solitary brush cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>bitter?</td>
</tr>
<tr>
<td>duodenum</td>
<td>L-cells</td>
<td>T1R2/T1R3, α-gustducin, PLCβ2</td>
<td>glucose, sucrose</td>
</tr>
<tr>
<td>ileum</td>
<td>solitary brush cells</td>
<td>T2Rs, α-gustducin, TRPM5</td>
<td>bitter?</td>
</tr>
<tr>
<td>Pancreatic ducts</td>
<td>enteroendocrine cells</td>
<td>T2R2/T1R3, α-gustducin, PLCβ2</td>
<td>glucose, sucrose</td>
</tr>
<tr>
<td>Testis</td>
<td>spermatozoa</td>
<td>α-gustducin</td>
<td>chemoattractants?</td>
</tr>
</tbody>
</table>
Sensory transduction in the enteroendocrine cell

Bertrand, Front. Neurosci, 2009
Спасибо за внимание!
PKD2L1 TRP channel is a sour receptor

Wild type

Pkd2l1-DT
ASIC family of H+-gated channels
Heterogeneity of taste bud population

- **NaCl**: Salty
- **Sucrose**: Sweet
- **Citric acid**: Sour
- **Bitter**: Bitter
- **Umami**: Umami

**Cell Types**:
- **Type I**: Basal cells
- **Type II**: Taste cells
- **Type III**: Sweet, Salty, Bitter, Umami, Sour