Tasty Genes: Diverse Receptors Mediate Distinct Taste Modalities

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Abstract

Receptor cells are responsible for the detection and transduction of external stimuli in our environment into internal sensory perception, such as chemical (taste and smell) and physical (temperature, sound, light, and mechanical) features. Taste is essential for guiding organisms to identify specific chemicals that potentially possess nutritious or noxious properties. In this review, I will discuss some of the significant findings from my lab in which we identified several classes of taste receptor cells (TRC's) critical for transducing the basic taste modalities (sweet, sour, salty, umami, bitter). Using specialized cell culture techniques, used in conjunction with rodent models, we have also identified several classes of receptor proteins underlying the molecular recognition and processing of these five senses. These classes include the T1R super family (sweet & umami), T2R's (bitter), PKD2L's (Sour), and more recently the EnaC's (salty), each of which are broadly expressed in TRC's. Our findings, and those of others, support the hypothesis that peripheral coding of taste modalities is broadly tuned via an 'across-fibre' pattern of coding. We conclude by discussion several challenges that remain to be addressed in taste signaling, such as how taste coding occurs beyond the periphery.

Introduction

Our sensory systems are tasked with the responsibility of providing an accurate representation of the external environment, allowing organisms to navigate and survive in a dynamic physical world. Mechanosensory, visual, and auditory senses allow organisms to detect physical properties in the environment, whereas olfactory and taste sensory systems enable us to detect chemical features of the environment. Although much has been learned about the auditory, visual, mechanosensory, and olfactory systems, little is known about how we taste chemical features in the environment. Rigorous research from the last 10 years has identified several classes of taste receptor cells (TRC) and receptor proteins necessary for taste transduction. This review will examine the significant findings that have emerged in the past decade and also highlight some of the questions that have yet to be answered in taste transduction.

Mammalian Taste Receptors

Mammals are capable of detecting a broad variety of chemical stimulants, which can be classified under five basic taste modalities: sweet, umami, sour, bitter, and salty (Chaudhari *et al*, 2010). Taste is essential to the survival of the individual to identify and consume the necessary nutritious elements such as amino acids and carbohydrates. The ability to taste sweet substances allows us to ingest the necessary saccharides essential for internal energy production. Salty taste ensures the necessary ingestion of ions such as Na+ and K+, whereas bitter and sour alert us to potentially noxious compounds and poisons. Taste sensation serves to draw the organism towards potentially "good" food items and to avoid potentially "bad" food sources. This modest sensory discrimination is evidenced by our inability to discriminate between chemical compounds within each sense. As a result, we are well equipped to discriminate between the senses rather than within. This in turn makes it easier for the agent to discriminate between what must be ingested and what must be avoided (Yarmolinsky *et al*, 2009, Huang *et al*, 2006).

Taste transduction in mammals occurs via specialized taste receptor cells selectively distributed on the surface of the tongue and palate. TRC are further assembled into taste buds; clusters consisting of 50-150 neuroepithelial cells, typically arranged in papillae structures embedded in the tongue surface. There are three types of papillae structures. Fungiform papillae are a set of taste buds located to in the anterior two third region of the tongue and typically consist of 1 or a few taste buds per papilla. Foliate papillae are located to the posterior lateral edges of the tongue, and contain hundreds of taste buds. Circumvallate papillae, located in the posterior end, contain thousands of taste buds (Yarmolinsky *et al,* 2009; Huang *et al*, 2006) . Contrary to the old notion of a "tongue map", which spatially designates specific areas of the tongue to specific taste modalities, all papillae structures contain receptors for detecting all five sense modalities (Lindemann, 1999). Fungiform papillae re innervated by the chorda tympani of the facial nerve, whereas foliate and circumvallate papillae are inverted by the glossopharyngeal nerve.

Taste Receptor Proteins

How do TRC tranduce chemical stimuli into the sensation of taste? Rigorous scientific evidence from my lab, and those of others, have identified several classes of membrane proteins responsible for detecting each of the five taste modalities. The following sections further discuss each taste modality with respect to its unique set of membrane receptor proteins.

Sweet

Detection of sweet tasting molecules not only enables organisms to detect sugar content within potential food items, but also activates higher order hedonic behavioral responses. This close association between sweet quality and pleasurable response is an illustration of how evolution has selected for the most fundamental source of energy. In 2000, we published a paper identifying putative taste receptors selectively in subsets of taste receptor cells of the tongue and palate (Fuller, 1974). Among these taste receptors is a modest class of G-protein coupled receptors (GPCRs) known as the T1R family. Previous research had identified a principal locus for sweet tasting in mice that influences responses to sweet chemicals (Fuller, 1974). Genetic linkage studies conducted by several groups identified the Sac gene as the T1R3 allele. We used engineered Sac mice expressing the T1R3 allele and found that this allele rescues sweet taste deficiency in Sac mice, suggesting that the T1R family may represent the sweet taste receptors. We then examined the expression pattern of T1R receptors and found three distinctive patterns of expression: (1) T1R1 + T1R3, (2) T1R2 + T1R3, and (3)

* This author wrote the paper for Biology 346: Molecular Neuroscience taught by Dr. Shubhik DebBurman

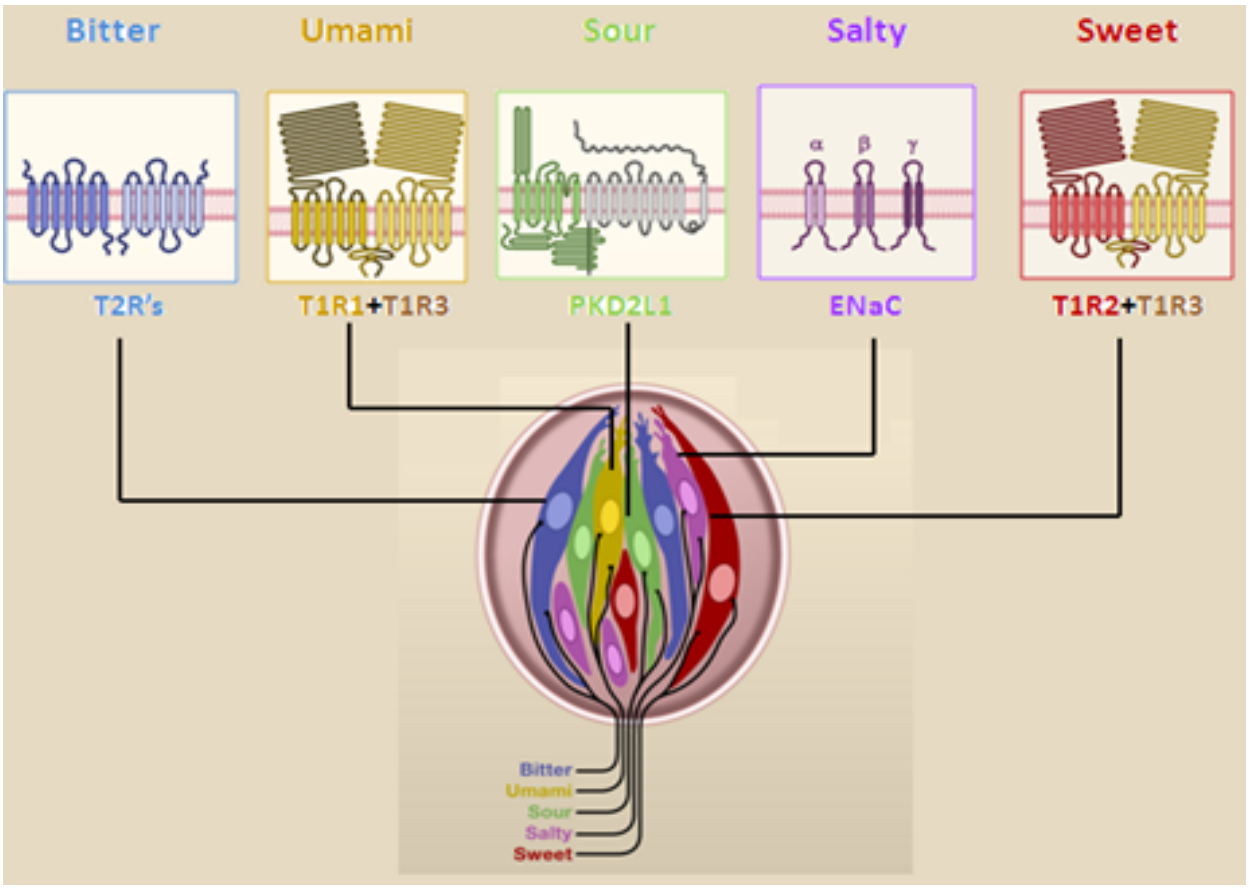

Figure 1: Basic Anatomy a Taste Receptor Cell:
Above figure illustrates an oversimplified labeled lined model of taste transduction in a taste receptor cell (TRC). Each cell expresses a unique set of specialized receptor proteins to which chemical taste binds, inducing a series of steps that code for a particular taste. [Note: Protein receptors are partially expressed in a single receptor cell and no one cell expresses all receptor proteins as shown in the above figure].

T1R3 expressing taste receptors cells11. We then showed that T1R2 + T1R3 (T1R2+3), but not (T1R1+3) or T1R3, co-expressing cells respond robustly to a variety of sweet compounds in a dose dependent manner. Further analysis showed that co-expression of T1R2+3 were necessary for sweet tastant response as neither T1R2 nor T1R3 expressed in isolation produced any response (Zhao *et al*, 2003).

Definitive proof that T1R2+3 are indeed the sweet receptor proteins came from Li *et al.* (2002) who used transgenic mice to evaluate responses to a variety of sweet tastants using combinations of T1R2 and T1R3 expression. T1R3 knock-out mice show significant ablation to sweet tastants (Damak *et al*. 2003). Interestingly, we also find that T1r2 or T1r3 KO mice show residual responses to extreme sugar concentrations. Nevertheless, T1r2 and T1r3 (T1R2+3) KO mice show complete loss to sweet sensation even at very high concentrations of sugar solutions. Recent evidence to support the importance of T1R2 and T1R3 receptors in mediating sweet sensation comes from studies done in felinae. Cats, long known to be sweet insensitive, have now been shown by Li *et al.* (2005) to have a natural deletion of the T1r2 gene.

Several studies (Xu *et al*. 2004; Jiang *et al*. 2005) have investigated how hundreds of sweet tasting compounds, from six carbon sugars to complex sweet tasting peptides, can bind only two receptor proteins. Evidence from such studies now suggests that different chemical compounds bind to unique regions of the T1R2+3 protein complex. This finding indicates that one complex can indeed respond to various unrelated chemical compounds to produce a similar response.

Umami

Borrowed from the Japanese vocabulary, umami describes the flavor typical of protein rich foods such as meats, seafood, vegetables, and cheese, which often induce a 'delicious' flavor. Several mammals are attracted to amino acid tastants such as glutamate. Humans, however, only respond to a monosodium-glutamate (MSG) and L-aspartate.

By applying similar techniques and logic utilized to identify and characterize sweet taste receptors, we also showed conclusively that T1R1+3 co-expression is necessary for detecting umami taste in mammals. Using transgenic KO mice as a model, we showed that elimination of either T1R1 or T1R3 (but not T1R2) diminishes responses to MSG and several other L-amino acids in mice (Zhao *et al*. 2003; Nelson *et al*. 2002). Several other investigators have provided evidence to support the hypothesis that T1R1+3 is the principal receptor for umami taste (Li *et al*. 2001).

Bitter

In addition to recognizing attractive tastants, mammals must also be able to recognize potentially harmful chemicals. This task may seem daunting considering the abundance of potentially harmful substances in the environment. Another challenge faced by bitter TRC is that the concentration threshold for detecting potentially noxious substances must -

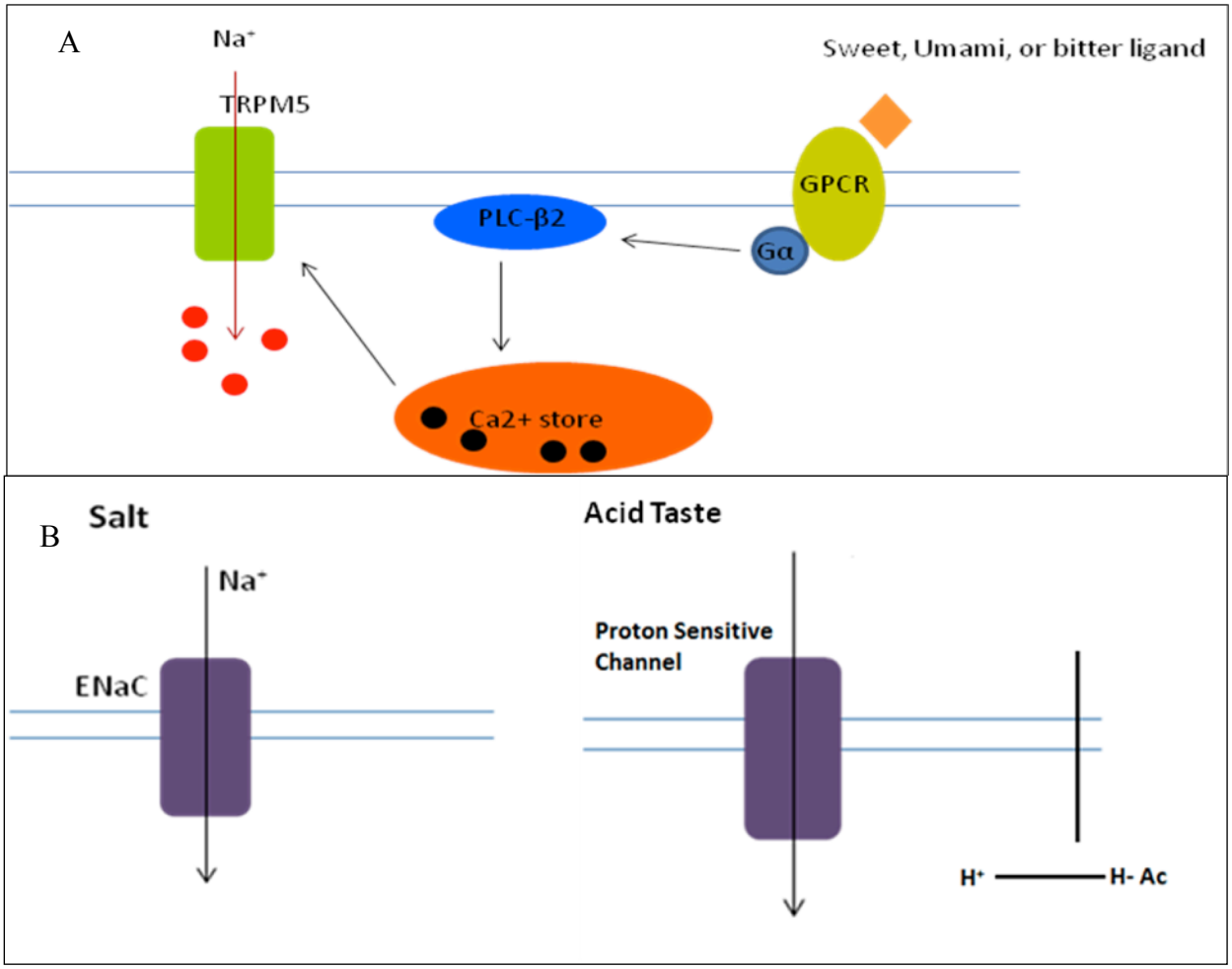

Figure 2: Intracellular Signaling Mechanism

A) shows the signaling mechanisms by which sweet, umami, and bitter tastants are transduced in TRC. The pathways for GPCRs (sweet, umami, and bitter) are unique from those of salty and sour tastants which seem to require a simpler direct route of activation.

be significantly lower than that required for detecting attractive substances (Chaudhari & Roper 2010).

Another significant finding to come from our lab was the identification of another unique family of GPCRs known as the T2R family, consisting ~40 structurally diverse trans-membrane proteins. Using a combination of behavioral, genetic, and physiological studies, we have shown that T2R receptor proteins are responsible for the detection of bitter tastants (Adler *et al*. 2000; Chandrashekar *et al*. 2000). To illustrate the role of T2Rs in bitter taste perception, we engineered mice expressing human T2R receptors for bitter transduction. More importantly, the finding that human T2Rs transfected into mice induce robust responses to novel bitter substa nces illustrates the evolutionary importance of bitter sensation across mammalian species.

Unlike sweet and umami taste receptors, T2Rs are almost all expressed in the same TRCs. Moreover, they do not overlap with sweet and umami TRCs (Adler *et al*. 2000). We interpreted this finding as a consequence of the evolutionary need to identify a broad range of bitter compounds without the ability to discriminate between individual bitter substances.

Sour

It has been proposed that sour sensing is mediated by PKD2L1, PKD2L3, and HCN1 receptor proteins (Huang *et al*. 2006). Inhibition of PKD2L1, using toxins specifically targeted to the membrane protein, has been shown to attenuate cellular responses to acid substances without hindering the functional properties of other receptor proteins. The precise mechanism for detecting acids has yet to be elucidated as current evidence has been based on genetic ablation studies where PKD2L1 is blocked by specific toxins. The need for KO studies is important if we are to learn the precise nature of acid sensing. Recently, Chandrashekar *et al*. (2009) has provided evidence that supports an acid sensing dependent mechanism for detecting CO_2. The ability to sense carbonation is dependent on PKLD2L1 receptors. Indeed, our lab has shown that genetic ablation of PKD2L1 receptors partially eliminates CO_2 sensing. We used Car4, an extracellular glycosylphosphatidylinositol (GPI)- anchored carbonic anhydrase, to function as the main CO_2 sensor.

Currently, it is believed that sour sensing occurs via proton sensing. Consistent with this hypothesis is the discovery of PKD2L1 receptors within the central canal of the spinal cord in mice, a finding that suggests that PKD2L1

senses acids via protons present within the mammalian body for pH regulation (Huang *et al*. 2006). Overall, these findings do indicate that non-GPCR mediated sensing of sour and salty taste is mediated via specialized membrane proteins rather than the action of simple ion channels.

Salty

The ability to sense salty foods is one of importance, yet it remains one of the least understood senses within the taste spectrum. Sodium is a major cation within many organisms and its presence is pervasive throughout the entire organ system. In humans and rodent models, the ability to sense Na+ is dependent on the internal concentration of Na+. When deprived of Na+, rats become particularly attracted to Na+ rich solutions, whereas, higher internal concentrations of Na induce aversive behavioral responses to Na+ solutions (Yarmolinsky *et al*. 2009).

Epithelial sodium channels (ENaCs) have very recently been proposed to be the potential Na sensing protein receptors. Evidence to support the role of ENaC comes from the finding that Cre-Lox transgenic mice with significant ablation of ENaCs to the tongue show little or no appetite for Na even at extreme deprivation levels (Chandrashekar *et al*. 2010). Indeed ENaC expressing cells are distinct from those expressing umami, sweet, and bitter receptors. The ability to study sodium sensing in rodent models is inhibited by the observation that ENaC KO mice and rats die within a few days after birth. This illustrates the significance of Na sensing not just at the level of taste but also for the maintenance of a stable internal environment.

Further investigations are necessary to unravel the precise mechanisms and pathways for Na sensing as it constitutes one of the essential needs for vertebrate survival.

Beyond the TR

Sweet, umami, and bitter taste are mediated by GPCRs, a class of proteins distinct from the ENaCs and PKD2L1s. At present, it is believed that T1R and T2R activation occurs as follows: G protein gustacin (Gα) is activated leading to the release of Gα subunits which then stimulates phospholipase (PLC-β2). Activation of phospholipase ultimately leads to the gating of the transient receptor protein (TRPM5). Evidence to support this comes from data showing that elimination of either TRPM5 or Gα in cell assays eliminates responses to sweet, umami, and bitter tastants but not salty or sour substances (Zhang *et al*. 2003). At best this model provides a somewhat clear picture of the intracellular mechanisms involved in taste transduction. More importantly, they give us the necessary clues to answer a debate that has long been unsettled in taste transduction research; does taste transduction occur via a labeled line mechanism of coding or via an across-fiber pattern of coding.

Several lines of evidence are now available that point to a labeled line model of taste coding (Tomchik *et al*. 2007). We recently, tested the hypothesis that taste coding in the periphery occurs via a labeled line model (Zhang *et al* 2003). As phospholipase KOs result in no response to sweet, umami, or bitter compounds, we reasoned that is indeed TRCs were broadly tuned to sweet, umami, and bitter taste. Subsequent restoration of phospholipase to a specific TRC (expressing T2R) should induce a response to all three taste modalities regardless of the fact that T2R expressing cells had been rescued. However, if TRC were tuned to a single modality, then restoration of phospholipase (to T2R cells only) should only rescue responses to bitter taste. We have shown that exclusive T2R -expressing phospholipase rescue in transgenic mice is limited to bitter tastants and not to sweet or bitter (Zhang *et al*, 2003).

In another classic experiment, Mueller *et al*. (2003) engineered mice that expressed an opioid receptor (receptor activated solely by a synthetic ligand: RASSL) in sweet and bitter TRCs. Animals expressing the RASSL in bitter TRC were found to be averted by activation of the ligand. Likewise, expression of RASSL in sweet TRC induced attraction to opioid agonist (Zhang *et al*. 2003). Together, these studies demonstrate tasting does indeed occur via a labeled line model of periphery coding.

A taste of things to come

Although we have made significant advances in understanding taste transduction, there is much that we do not fully understand and appreciate about taste transduction and perception. Prior knowledge about olfaction, vision and other senses illustrates the importance of lateral inhibition at the periphery. How then do TRCs communicate with one another? Is lateral inhibition necessary at all for taste transduction to occur?

The greatest gaps in knowledge concern the central representation of taste. How is taste information processed in the central nervous system? More importantly also, what is the role played by other centers in regulated feeding behavior. How does olfaction combine with taste information to produce the perception of flavor? Visual cues such as color can make a significant difference in whether or not we choose to consume potential food items.

Acknowledgements

I would like to thank professor Shubhik DebBurman for his outstanding leadership and guidance throughout the semester and the rest of the Bio 346 class for being a great family.

References

1. Chaudhari, N., & Roper, S. D. (2010). The cell biology of taste. Journal of Cell Biology, 190(3), 285-296.

2. Yarmolinsky DA, Zuker CS, & Ryba NJ. (2009). Common sense about taste: from mammals to insects. Cell. 139(2), 234-44.

3 .Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Trankner D, Ryba NJ, & Zuker CS. (2006). The cells and logic for mammalian sour taste detection. Nature. 442(7105), 934-8.

4. Lindemann, B. (1999). Receptor seeks ligand: On the way to cloning the molecular receptors for sweet and bitter taste. Nature Medicine, 5(4), 3814.

5. Chandrashekar J, Kuhn C, Oka Y, Yarmolinsky DA, Hummler E, Ryba NJ, & Zuker CS. (2010). The cells and peripheral representation of sodium taste in mice. Nature. 464(7286), 297-301.

6. Chandrashekar J, Yarmolinsky D, von Buchholtz L, Oka Y, Sly W, Ryba NJ, & Zuker CS. (2009). The taste of carbonation. Science (New York, N.Y.). 326(5951), 443-5.

7. Chandrashekar J, Hoon MA, Ryba NJ, & Zuker CS. (2006). The receptors and cells for mammalian taste. Nature. 444(7117), 288-94.

8. Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, & Zuker CS. (2003). The receptors for mammalian sweet and umami taste. Cell. 115(3), 255-66.

9. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, & Zuker CS. (2002). An amino-acid taste receptor. Nature. 416(6877), 199-202.

10. Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, & Zuker CS. (2000). A novel family of mammalian taste receptors. Cell. 100(6), 693-702.

11. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, & Zuker CS. (2001). Mammalian sweet taste receptors. Cell. 106(3), 381-90.

12. Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS, & Ryba NJ. (2005). The receptors and coding logic for bitter taste. Nature. 434(7030), 225-9.

13. Zhang Y, Hoon MA, Chandrashekar J, Mueller K, Cook B, Wu D, Zuker CS, & Ryba NJ. (2003). Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell. 112(3), 293-301.

14. Zuker CS. (2002). Neurobiology: a cool ion channel. Nature. 416(6876), 27-8.

15. Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, & Ryba NJ. (2000). T2Rs function as bitter taste receptors. Cell. 100(6), 703-11.

16. Hoon, M. A., & Adler, E. (1999). Putative mammalian taste receptors: A class of taste-specific GPCRs with distinct topographic.. Cell, 96(4), 541.

17. Xia, L., Weihua, L., Hong, W., Jie, C., Kenji, M., Liquan, H., & ... Flint, J. (2005). Pseudogenization of a Sweet-Receptor Gene Accounts for Cats' Indifference toward Sugar. PLoS Genetics, 1(1), 27-35. doi:10.1371/journal.pgen.0010003

18. Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., & ... Margolskee, R. F. (2003). Detection of Sweet and Umami Taste in the Absence of Taste Receptor T1r3. Science, 301(5634), 850-853.

19. Fuller (1974). Single Locus Control of Saccharin Preference in Mice. Journal of heredity, 65, 33-36.

20. Wei, H., Yasumatsu, K., Varadarajan, V., Yamada, A., Lem, J., Ninomiya, Y., & ... Damak, S. (2004). Umami Taste Responses Are Mediated by α-Transducin and α-Gustducin. Journal of Neuroscience, 24(35), 7674-7680. doi:10.1523/JNEUROSCI.2441-04.2004

21. Heath, T. P., Melichar, J. K., Nutt, D. J., & Donaldson, L. F. (2006). Human Taste Thresholds Are Modulated by Serotonin and Noradrenaline. Journal of Neuroscience, 26(49), 12664-12671. doi:10.1523/JNEUROSCI.3459-06.2006

22. Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C, & Li X. (2008). Molecular mechanism for the umami taste synergism. Proceedings of the National Academy of Sciences of the United States of America. 105(52), 20930-4.

23. Xu H, Staszewski L, Tang H, Adler E, Zoller M, & Li X. (2004). Different functional roles of T1R subunits in the heteromeric taste receptors. Proceedings of the National Academy of Sciences of the United States of America. 101(39), 14258-63.

24. Jiang P, Cui M, Ji Q, Snyder L, Liu Z, Benard L, Margolskee RF, Osman R, & Max M. (2005). Molecular mechanisms of sweet receptor function. Chemical Senses. 30, i17-8.

25. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, & Shirazi-Beechey SP. (2007). T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. Proceedings of the National Academy of Sciences of the United States of America. 104(38), 15075-80.

26. Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, & Zuker CS. (1999). Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell. 96(4), 541-51.

27. Janssen S, Laermans J, Verhulst PJ, Thijs T, Tack J, & Depoortere I. (2011). Bitter taste receptors and α-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. Proceedings of the National Academy of Sciences of the United States of America. 108(5), 2094-9.

28. Brockhoff A, Behrens M, Niv MY, & Meyerhof W. (2010). Structural requirements of bitter taste receptor activation. Proceedings of the National Academy of Sciences of the United States of America. 107(24), 11110-5.

29. Tizzano M, Gulbransen BD, Vandenbeuch A, Clapp TR, Herman JP, Sibhatu HM, Churchill ME, Silver WL, Kinnamon SC, & Finger TE. (2010). Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals. Proceedings of the National Academy of Sciences of the United States of America. 107(7), 3210-5.

30. Wu SV, Rozengurt N, Yang M, Young SH, Sinnett-Smith J, & Rozengurt E. (2002). Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. Proceedings of the National Academy of Sciences of the United States of America. 99(4), 2392-7.

31. Xiaodong, L., Staszewski, L., Hong, X., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. Proceedings of the National Academy of Sciences of the United States of America, 99(7), 4692.

32. de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA, & Simon SA. (2008). Food reward in the absence of taste receptor signaling. Neuron. 57(6), 930-41.