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Review

Taste perception and coding in the periphery

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Abstract. Recent identification of taste receptors and their downstream signaling molecules, expressed in taste receptor cells, led to the understanding of taste coding in the periphery. Ion channels appear to mediate detection of salty and sour taste. The sensations of sweet, umami and bitter taste are initiated by the interaction of sapid molecules with the G-protein-coupled receptors T1Rs and T2Rs. Mice lacking either PLCβ2 or TRPM5 diminish behavioral and nerve responses to sweet, umami and bitter taste stimuli, suggesting that both receptor families converge on a common signaling pathway in the taste receptor cells. Nevertheless, separate populations of taste cells appear to be uniquely tuned to sweet, umami and bitter taste. Since PLCβ2-deficient mice still respond to sour and salty stimuli, sour and salty taste are perceived independent of bitter, umami and sweet taste. In this review, the recent characterization of the cellular mechanisms underlying taste reception and perception, and of taste coding in the periphery will be discussed.

Keywords. Taste, ion channels, G-protein-coupled receptors, signal transduction, taste coding.

Introduction

The taste sensory system plays a critical role in the life and nutritional status of organisms. It is responsible for detecting various compounds that are noxious or toxic, and that provide caloric energy. Humans can detect and discriminate between sweet, bitter, sour, salty and umami stimuli [1]. The sense of taste also evokes responses that range from innate behavioral actions such as aversion and attraction to food sources, to the pleasure of food consumption. Although the initial step in taste perception takes place at the specialized cells, taste receptor cells, in the oral space, those responses are substantially governed by the neuronal activities in the brain. Bitter is the main taste modality evoking aversive and displeasing responses. In contrast, humans are attracted by sweet and umami tastants, and express pleasure for them. Generally speaking, salty and sour tastes are the appetitive and aversive modalities, respectively, however, depending on their concentrations, those tastes also evoke the converse responses. On the other hand, a wide variety of organisms possess the ability to predict future events on the basis of relevant sensory cues, which is emblematic of associative learning. The phenomenon can be conveniently studied with acquisition and extinction of memory of conditioned taste aversion (CTA), in which mammals learn to reject an attractive tastant (conditioned stimulus) if this tastant is associated with subsequent visceral malaise (unconditioned stimulus) [2]. Therefore, the gustatory system provides us with a precious model to understand the molecular, cellular and system mechanisms underlying cognition, behavioral and emotional responses, learning, and also the interaction of emotion with cognition and memory.

To decipher the underlying molecular, cellular and system mechanisms, it is first necessary to understand taste perception and coding in the periphery, and then to clarify the neuronal circuitries which process and integrate the information of aversive and attractive taste modalities. Taste perception in mammals is mediated by specialized

epithelial cells (taste receptor cells) that are arranged in taste buds. The cells express taste receptors responsible for detecting sweet, bitter, salty, sour and umami stimuli [1] (Fig. 1a). Thus, taste receptor cells perceive chemical signals and produce changes in membrane potential and/or intracellular free calcium concentration which evoke neurotransmitter release onto gustatory afferent nerve fibers. Then, the afferent fibers innervating the receptor cells transmit taste information such as intensity and quality to the gustatory cortex through synapses in the brain stem and thalamus [3]. Taste buds in the fungiform papillae located at the anterior two-thirds of the tongue and in the palate are innervated by the ganglion neurons in branches of the facial nerve, the chorda tympani and greater superficial petrosal nerve, respectively. Taste buds in the foliate and circumvallate papillae located at the posterior tongue are innervated by the glossopharyngeal nerve. The superior laryngeal nerve, a branch of the vagus nerve, innervates the taste buds in the epiglottis [3]. Each ganglion neuron contacts multiple taste receptor cells within a taste bud and from different taste buds, and relays taste information to the neurons in the solitary tract nuclei of the medulla. From the solitary tract nuclei, taste information is transferred to the neurons in the pontine parabrachial nuclei, then to the thalamus, and then to the gustatory cortex. Anatomical and physiological data elegantly suggested that gustatory information is processed and integrated by those nuclear relays and their connecting pathways in the brain [4–11], although the exact dimensions and internal organization for each taste modality remain unclear. Nevertheless, we can definitely learn many things from previous electrophysiological data of single neurons, which indicated how individual taste-responsive neurons process and integrate taste information, while changing their firing rates in response to various taste compounds.

In contrast, taste receptor proteins triggering signal transduction events remained elusive for a long time. More recently, taste receptor proteins, which are mainly mounted on the apical end of taste cells, were identified by means of electrophysiology, molecular biology, genetic approaches and also screening the mammalian genome sequence of the chromosome map locations associated with behavioral taste defects. Discovery of taste receptors led to the clarification of molecular and cellular mechanisms of taste perception in taste receptor cells. Furthermore, by employing taste receptor genes as molecular probes, the logic of taste coding in the periphery was also deciphered among particular taste modalities. In this review, the recent characterization of the molecular and cellular mechanisms of taste perception will be discussed, and taste coding in the periphery will be considered.

Mechanisms of salty and sour taste transduction

Salt perception plays a role in incorporating NaCl and other salts, and maintaining ion and water homeostasis, but the taste becomes unpleasant and aversive when too strong. Sour perception is devoted to the rejection of spoiled food, immature fruits or toxic substances, although the taste is acceptable when mild. Ion channels appear to mediate detection of salty and sour taste [12] (Fig. 1b).

NaCl salt taste is initiated by current flowing into the taste cells through cation channels located in the apical membrane [13]. The basolaterally located cation channels may also contribute to salt taste reception, following salt diffusion through paracellular pathways [14]. Na^+ influx through the cation channels elicits membrane depolarization [13], leading to production of action potentials that result in neurotransmitter release onto an afferent nerve fiber [12]. Action potentials involve activation of voltage-gated Na^+ and Ca^{2+} channels, which elicits inward Na^+ and Ca^{2+} currents and contributes to the depolarization phase, and activation of voltage-gated K^+ channels, which contributes to the repolarization and after-hyperpolarization phases [12, 15–17]. Thus, action potentials are expected to elicit Ca^{2+} inflow and subsequently synaptic exocytosis.

Like other Na^+-absorbing epithelia, Na^+ ions, transported into the taste cells substantially through apically localized cation channels [12], are extruded by basolaterally located Na^+-K^+-ATPases [18–21], followed by Cl^- transport from the apical to the basolateral side possibly via a paracellular route. Thus, it is likely that the selective anion permeability of the tight junctions contributes to the distinct taste of various sodium salts [22, 23]. Importantly, membrane potentials and $[Na^+]_i$, which are affected and maintained by Na^+ influx through apically localized cation channels and Na^+ efflux through Na^+-K^+-ATPases, regulate repetitive firing of action potentials, governed by the voltage-gated channels.

Chorda tympani taste nerve responses to NaCl can be dissected pharmacologically into amiloride-sensitive and amiloride-insensitive components [24–27]. When NaCl is applied to the anterior tongue surface, the epithelial Na^+ channel (ENaC) blocker amiloride significantly suppressed the transepithelial current [21], accompanied by the inhibition of part of the chorda tympani nerve response to NaCl [21]. The results importantly suggest that ENaC functions as a transducer for NaCl taste in taste receptor cells [28]. Chorda tympani nerve recording to NaCl also shows an amiloride-insensitive response [29] that is profoundly dependent on anion [23], and part of which is thought to involve a paracellular pathway [14]. Thus, Na^+ ions may also penetrate the epithelial surface via the tight junction, and enter taste receptor cells through the basolaterally located, amiloride-insensitive channels. Single-

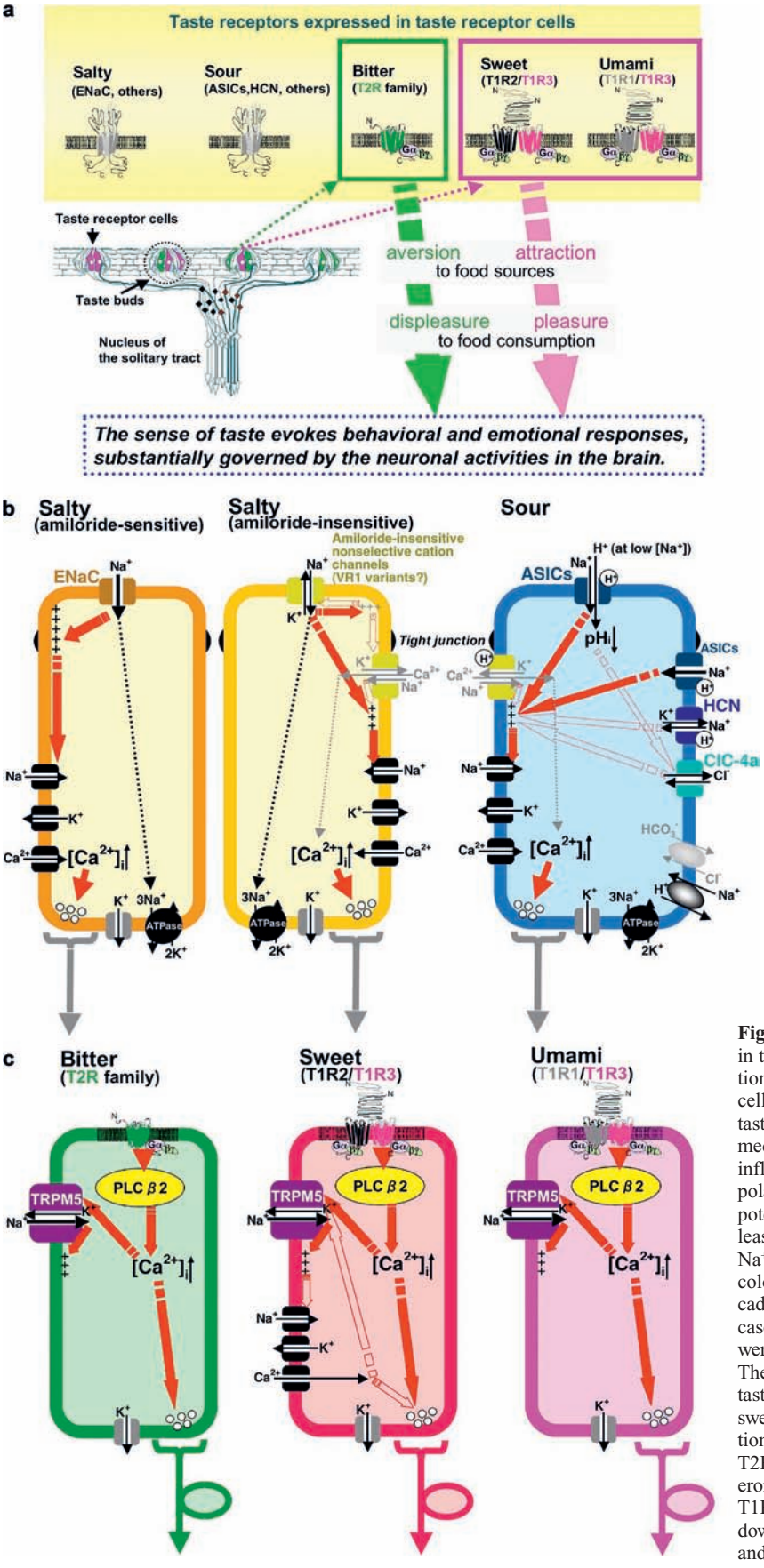

Figure 1. Taste receptors and signal transduction in taste receptor cells. (*a*) Schematic representation of taste receptors expressed in taste receptor cells. (*b*) The proposed models of salt and sour taste signal transduction. Ion channels appear to mediate detection of salt and sour taste. Cation influx through the channels elicits membrane depolarization, leading to the production of action potentials, which results in neurotransmitter release onto an afferent nerve fiber. Voltage-gated Na^+, K^+ and Ca^{2+} channels are denoted as black-colored channels. The order of signaling cascades was denoted using red arrows. The putative cascades, proposed by their molecular functions, were also denoted using red-lined arrows. (*c*) The proposed models of bitter, sweet and umami taste signal transduction. The sensations of bitter, sweet and umami taste are initiated by the interaction of sapid molecules with GPCRs such as the T2Rs, the T1R2/T1R3, and the T1R1/T1R3 heteromers, respectively. Ligand binding to T2Rs, T1R2/T1R3, and T1R1/T1R3 activates common downstream signaling molecules such as PLCβ2 and TRPM5 in taste receptor cells.

unit studies show that the amiloride-sensitive responses are associated with N fibers that respond specifically to Na salts, suggesting that N fiber responses are ascribed to taste receptor cells expressing ENaC that is blocked by amiloride and selectively conducts Na^+ among monovalent cations [24, 25]. Amiloride-insensitive responses are associated with H-fibers that respond to Na^+, K^+, NH_4^+ and other cations, suggesting that H fiber responses are ascribed to taste receptor cells that contain nonselective-cation pathways serving as a transducer for cation taste responses [24, 25].

The inward flux of Na^+ through ENaC plays an important role in triggering amiloride-sensitive Na^+ salt taste transduction (Fig. 1b). This is supported by single-cell patch clamp recordings directly showing the existence of amiloride-sensitive Na^+ channels in taste receptor cells [13]. ENaC is an apically localized, heterotetrameric channel composed of three homologous subunits of α, β and γ, and displays the following characteristics: high sensitivity to amiloride (Ki = about 0.1 μM), low single-channel conductance (4–7 pS) and high selectivity for Na^+ over K^+ [30]. Indeed, mammalian taste cells possess amiloride-sensitive Na^+ channels sharing electrophysiological and pharmacological properties with ENaC channels [31]. In addition, all three ENaC subunits are reported to be expressed in most of taste cells of fungiform papillae and in about half of the taste cells of foliate and vallate papillae [32, 33]. The expression levels of ENaC in cells of vallate papillae seem to be significantly lower than in cells of fungiform papillae, especially for β and γ subunits [33]. Approximately 65% of the fungiform taste cells exhibit functional amiloride-sensitive Na^+ currents. However, the taste cells of vallate papillae are completely insensitive to amiloride, suggesting that ENaC channels expressed in taste cells of vallate papillae are located in cytoplasmic compartment, but not in the apical membrane [33]. ENaC is a spontaneously open channel, and its degradation is tightly controlled by a Nedd4-dependent pathway [34]. Therefore, in taste receptor cells, the appearance and magnitude of the apical Na^+ influx through ENaC might be regulated by the rates of transcription, biogenesis, apical sorting and degradation of ENaC subunits. Of note, the elevated levels of aldosterone, known to increase Na^+ transport in tight epithelia [34] as well as the amiloride sensitivity of the NaCl taste response [35], resulted in increased immunoreactivity for β and γ subunits in apical regions of taste buds in all papillae, with the most striking effects in vallate papillae [33]. Accordingly, aldosterone treatment increased the amplitude of the amiloride-sensitive current in fungiform taste cells, and induced amiloride sensitivity in vallate taste cells [33]. Therefore, the correlation between the induced changes in amiloride-sensitive currents and ENaC expression suggests that ENaC is responsible for functional amiloride-sensitive Na^+ currents in taste receptor cells, and thus plays substantial roles in triggering amiloride-sensitive Na^+ salt taste transduction.

In taste receptor cells from many mammalian species, amiloride-insensitive cation pathways for Na^+ influx appear to elicit a significant part of the chorda tympani taste nerve responses and most of the glossopharyngeal taste nerve responses. However, little is known regarding the pathways triggering amiloride-insensitive Na taste transduction. A recent report indicated that an apical amiloride-insensitive cation pathway in taste receptor cells is modulated by cetylpyridinium chloride (CPC) [36]. More recently, chorda tympani taste nerve responses of vanilloid receptor-1 (VR1)-deficient mice suggested that the CPC-sensitive, amiloride-insensitive cation channel (the salt taste receptor) is a constitutively active non-selective cation channel derived from the VR1 gene [29]. Thus, VR1 knockout mice have diminished the nerve responses mediated by functional amiloride-insensitive salt taste receptors, but not the responses to sweet, bitter and sour taste. However, the channel properties deduced in the study showed distinct pharmacological and biophysical characteristics, including external pH sensitivity, from both the vanilloid receptors expressed in trigeminal ganglion neurons [37] and the recombinant VR1 channels expressed in heterologous cells [38–40]. Therefore, it is suggested that a variant of the VR1 receptor may function as the non-sensitive cation channel in amiloride-insensitive salt taste transduction in taste receptor cells. Generally, VR1 is permeable to Na^+, K^+ and Ca^{2+} but relatively selective for Ca^{2+} (P_{Ca}/P_{Na} = 10), and has an outwardly rectifying current-voltage relationship. Divalent cation permeability of VR1 depends on a single aspartic acid residue in the pore region of the channel. VR1 is activated by vanilloid compounds as well as by multiple other stimuli, including moderate heat (>43 °C) and low pH (<5.9) [38–40]. Activation of VR1 occurs in a voltage-dependent manner upon depolarization [41]. Increases in temperature result in a gradual leftward shift of the voltage-dependent activation curves, as do stimuli with capsaicin [41]. It is now of critical importance to understand the molecular nature of VR1 variants expressed in taste receptor cells, the subunit composition of the cation channel including VR1 variants, and also their localization in the cells. Since VR1 variants might not have apical sorting signals by analogy with VR1, they might also be expressed in the basolateral membrane. In such a case, there may be differences between the open probabilities of the apically located and basolaterally located VR1 variants, depending on potential differences between the mucosal and interstitial sides of taste buds that resemble Na^+-absorbing tight epithelia. Thus, it is speculated that stimulation with Na and K salts may first elicit membrane depolarization by the influx of Na^+ and K^+ ions through apically located open VR1 variants. Second, the membrane depolarization might increase the open prob-

ability of basolaterally located VR1 variants which are permeable not only to Na^+ and K^+ penetrating via the tight junction, but also to Ca^{2+} in the interstitial side, leading to further membrane depolarization and an increase in $[Ca^{2+}]_i$ (Fig. 1b). Therefore, the characterization of the pharmacological and biophysical properties of the VR1 variants cloned from taste receptor cells, including current-voltage relationship, external pH dependence, Ca^{2+} permeability and ion selectivity for monovalent cations, may help define the molecular and cellular mechanisms responsible for amiloride-insensitive salt taste transduction.

Protons appear to be the primary sour taste stimuli since acid-elicited sour taste is proportional to proton concentration. Sour stimuli elicit action potentials from taste cells in a dose-dependent manner. Proton-gated channels, proton-conducting channels as well as pH-dependent ion exchangers in taste cells are believed to be involved in sour taste transduction or to act as sour taste receptors, while eliciting a robust sour sensation at a pH of <5 (Fig. 1b). Because protons possess significant permeability through the tight junctions and permeate through cells, they potentially modify the activities of both apically localized and basolaterally localized ion transporters extracellularly and intracellularly. Generally, upon acid stimulation, proton-activated or -modulated channel activities elicit membrane depolarization directly via current flow through the channel. Alternatively, pH-regulated ion exchangers or transporters modulate the intracellular concentration of ions such as Na^+, K^+ and Cl^-, and thus change the equilibrium potential for those ions, leading to membrane depolarization via current flow through the already opened channels. Membrane depolarization may be associated with the production of action potentials through activation of voltage-gated channels, resulting in the release of neurotransmitter onto an afferent nerve fiber. Thus, a variety of molecular mechanisms have been proposed to mediate sour taste transduction. Those include the acid-sensing ion channel (ASIC) of the ENaC/DEG family [42, 43], the hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) [44], the apical K^+ channel [45], the amiloride-sensitive ENaC [46] and the NPPB-sensitive Cl^- channel [47, 48]. Sour taste seems to result from integration of multiple pathways. Nevertheless, the contributory role of those channels in triggering sour taste transduction remains elusive.

Among those, recent results clearly demonstrated that the acid stimuli of taste receptor cells induce the large depolarizing current, which shares general properties with currents mediated by ASICs, accompanied by an increase in $[Na^+]_i$ [42]. The results strongly suggest that ASICs or ASIC-like channels, which are located in both the apical and basolateral membrane, are activated by extracellular protons and selectively conduct Na^+ over K^+, may play an important role in triggering sour taste transduction in taste receptor cells. Several subunits, such as ASIC1, ASIC2a, ASIC2b and ASIC4, which possess distinct pH sensitivity and modifying effects, are expressed in taste receptor cells. Judging from pharmacological properties and pH sensitivity, the acid-activated currents in taste receptor cells are similar to those of ASIC2a and the ASIC2a/ASIC2b heteromer [43]. However, recent data on ASIC2-deficient mice show unaltered behavioral and electrophysiological responses to sour stimuli in the mutant mice [49]. Therefore, both recording from taste cells, in which the other ASIC subunits have been deleted, and characterizing the subunit composition and molecular nature of ASIC channels expressed in taste cells will be necessary to understand the role of ASICs in sour transduction.

On the other hand, the acid-induced current may represent the additional contribution of other acid-regulated channels unrelated to ASICs, such as HCN1 and HCN4. Although HCN is mainly located in the basolateral membrane and is permeable to Na^+ and K^+, acids penetrating through the tight junctions appear to lower the threshold for activation of HCN conductance. Thus, extracellular proton-activated HCN channels appear to elicit an inward current at resting potentials with strong correlation between the presence of HCN currents and response to sour stimulus [44]. Therefore, HCN might also participate in sour transduction. In addition, as mentioned above, taste receptor cells are reported to express VR1 and/or VR1 variants [29, 50], which are permeable to Na^+, K^+ and Ca^{2+}. At body temperature, extracellular acidification stimulates VR1 channel activity [38], dependent upon a specific extracellular glutamate residue within the channel. Thus, the molecular nature of the channel derived from the VR1 gene needs to be further addressed to investigate whether the channel activity is involved in sour taste transduction.

More recent data interestingly revealed that ClC-4, a voltage-gated chloride channel, and ClC-4a, its novel splice variant, are selectively expressed in taste receptor cells, compared with non-gustatory lingual epithelium [51]. Cl^- currents appear in a subset of excitable taste cells after postnatal day 8, which may correlate with the maturation step of taste bud cells [52, 53]. Although several classes of Cl^- channels are reported to be expressed in taste receptor cells, the specific functions of taste cell-associated chloride channels are still not well understood [54–56]. ClC-4a is a novel isoform of ClC-4 that skipped an exon near the 5′-end, resulting in the deletion of the N-terminal 60 amino acid residues. In comparison with ClC-4 [57], ClC-4a displayed reversed pH sensitivity, suggesting that the cytoplasmic N-terminal segment is involved in pH dependence [51]. Changes in intracellular pH may be involved in the sour transduction mechanism, since acid stimulation decreases intracellular pH in taste receptor cells and afferent nerve responses correlate with

the intracellular pH [58–60]. Mouse ClC-4 channels are open in the extracellular pH range of 7.5 to 6.5, and begin to close as the pH falls from 6.5. In contrast, ClC-4a is closed at pH 7.5 and 6.5, but open at lower pH [51]. Thus, it is expected that if $[Cl^-]_i$ is low, the acidification-dependent closure of ClC-4 may cause membrane depolarization. Conversely, if $[Cl^-]_i$ is high, dependent upon the activities of ion exchangers and transporters, the acidification-dependent closure of ClC-4 may cause membrane hyperpolarization. In contrast, it is expected that if $[Cl^-]_i$ is maintained low and high, the acidification-dependent opening of ClC-4a may cause membrane potential to hyperpolarize and depolarize, respectively. Thus, it may be possible that both ClC-4 and ClC-4a are involved in membrane depolarization, dependent on $[Cl^-]_i$ and the resting membrane potential. Therefore, they might participate in initiation of action potentials, in addition to their contributions to setting of the resting potential and modulation of action potential waveforms. It is now important to clarify the relative expression levels between ClC-4 and ClC-4a in taste receptor cells of fungiform, foliate and vallate papillae, their current characteristics in taste receptor cells, and the mechanisms maintaining $[Cl^-]_i$ by means of ion exchangers and transporters. Those data will help in understanding the contributory roles of these interesting Cl^- channels in sour transduction. Along these lines, sour taste transduction appears to result from the integration of multiple pathways. Identification of a key molecule triggering sour transduction may require both electrophysiological recording and imaging of $[Na^+]_i$, $[Cl^-]_i$, $[Ca^{2+}]_i$ and $[H^+]_i$ from taste cells, in which these channels have been systematically deleted one by one or in combinations, accompanied by the recordings from afferent nerve fibers.

Mechanisms of bitter, sweet and umami taste transduction

Bitter and sweet/umami are the main taste modalities evoking aversive and attractive responses, respectively. The sensations of bitter, sweet and umami tastes are initiated by the interaction of sapid molecules with G-protein-coupled receptors (GPCRs) in the apical membranes of taste receptor cells. Two families of mammalian taste receptors, T1Rs and T2Rs, have been implicated in sweet, umami and bitter detection [1, 61, 62] (Fig. 1c).

Screening of the genome databases led to the discovery of a novel gene family of GPCRs (T2Rs), some of which are located within chromosomal loci associated with bitter taste defects [63, 64]. It is reported that there are 36 intact T2R genes in mice and 25 genes in humans [65, 66]. Ongoing are studies addressing whether all T2Rs are true bitter taste receptors, and how a limited number of T2Rs can permit the perception of thousands of different bitter compounds. However, at present, several lines of evidence indicate that some T2Rs are functional bitter receptors both *in vivo* and *in vitro*. First, functional assays in heterologous cells expressing T2R transgenes identified some bitter compounds, but not sweet/umami tastants, as agonists for T2Rs. mT2R5 and rT2R9 respond to the toxic bitter substance cycloheximide [67, 68]. hT2R10 and hT2R16 respond to strychnine and β-glucopyranosides [68], respectively. hT2R38 responds to compounds such as phenylthiocarbamide (PTC) and propylthiouracil (PROP), which contain the N-C=S moiety [69]. hT2R14 is activated by naphthoic acid, picrotin, picrotoxinin, piperonylic acid, sodium benzoate and (–)-a-thujone, showing unique broad tuning towards a variety of structurally diverse bitter compounds [70]. Second, in mice, strains with impaired cycloheximide tasting have polymorphisms in the mT2R5 receptor, leading to less responsiveness to cycloheximide [67]. In humans, sequence variants in hT2R38 correlate with differences in bitterness recognition of PTC in humans [71], accompanied by differences in the ability of the receptor variants to detect PTC in heterologous, functional assays [69]. Finally, mT2R5-deficient mice do not detect cycloheximide, while retaining the ability to detect other bitter substances [72]. Thus, it is likely that subsets of T2Rs may function as narrowly tuned bitter receptors that bind to a few ligands, or receptors that bind to several bitter compounds containing the specific moiety, whereas a part of them could be specific but broadly tuned bitter receptors. By analogy with other GPCRs that contain short extracellular amino-termini, it is predicted that the residues important for ligand-binding specificities are located in the transmembrane domains and also in the intervening extracellular loops. At present, correlation between functions and polymorphisms in the T2R genes between strains provides limited information about the sites responsible for ligand binding [73, 74]. However, in ongoing evolutionary diversification of T2R receptors, regions or even single amino acid residues which are conserved among distant species may indicate the sites responsible for the general function of the receptor. In contrast, regions that change among closely related species or individuals within a species may be involved in fine tuning the action of the receptor, such as altering the ligand-binding region or affecting interactions with the corresponding G proteins, leading to an enhanced or reduced efficiency of downstream signal transduction [65, 75]. Thus, systematical functional assays with analyses of structure-function relationships and evolutionary diversification patterns may help in understanding the molecular basis underlying the narrowly or broadly tuned characteristics for ligand binding, which reveals how a small number of T2R genes can perceive thousands of structurally diverse bitter compounds.

The T1R receptor family contains three genes and belongs to the class C family of GPCRs, which possess a large N-

terminal extracellular domain and are known to function as either homodimers or heterodimers [76–82]. The T1R receptor family (T1R1, T1R2 and T1R3) generates at least two heteromeric receptors: T1R1/T1R3 and T1R2/T1R3 (Fig. 1c). The mouse T1R1/T1R3 heteromer appears to act as a sensor for many L-amino acids with similar affinity, whereas the human T1R1/T1R3 heteromer selectively senses L-glutamate over other amino acids [83, 84]. The discrepancy appears to be derived from the differences between human and rodent T1R1 subunits, since combination of human T1R1 and mouse T1R3, expressed in heterologous cells, became more responsive to L-glutamate than to other amino acids and than did mouse T1R1/T1R3 receptor. Thus, human and rodent T1R1/T1R3 receptors display selectivity and sensitivity differences that mimic differences in amino acid-mediated taste responses between human and rodent [84]. Furthermore, T1R1/T1R3 receptor activity is substantially enhanced by inosine monophosphate (IMP), known to potentiate umami taste *in vivo* [83, 84]. The T1R1/T1R3 receptor also responds to L-AP4, known as an agonist for the metabotropic glutamate receptors (mGluRs) and an umami-tasting compound [83, 84]. These results suggest that the T1R1/T1R3 heteromer functions as an umami receptor. Accordingly, mice lacking either T1R1 or T1R3 have diminished behavioral and nerve responses to glutamate and other L-amino acids [85]. Thus, T1R1/T1R3 heteromers are devoted to the detection of glutamate in human and many L-amino acids in rodents, although the additional involvement of other receptors, such as mGluR4t, a truncated variant of the metabotropic glutamate receptor, is considered in a full explanation of umami taste transduction [86–88].

The T1R2/T1R3 heteromers appear to function as taste receptors for natural sugars and artificial sweeteners [82–85]. T1R2/T1R3 double knockout mice do not display behavioral and nerve responses to sweet stimuli, supporting the function of T1R2/T1R3 as a sweet receptor [85]. However, it remains unclear how the single T1R2/T1R3 receptor can recognize a large collection of diverse chemical structures of sweeteners such as carbohydrate, some amino acids, proteins and synthetic sweeteners. The distinct ligand specificities of T1R1/T1R3 and T1R2/T1R3 receptors imply that T1R1 and T1R2 play more substantial roles in ligand binding of umami and sweet taste receptors than does T1R3. Accordingly, a recent study indicated that mice carrying human T1R2 transgene on the T1R2 knockout background display sweetener taste preference similar to those of humans [85]. Polymorphisms in the receptor genes between mouse strains and differences in human and rodent sweet taste receptors in terms of the ligand specificity, G protein-coupling efficiency and sensitivities to inhibitors are useful to delineate the sites within the receptor which are responsible for binding of ligands and inhibitors and coupling with G-proteins [89–94]. Thus, by employing domain swapping between human and rodent receptors in combination with site-directed mutagenesis, recent reports demonstrated the different functional roles of T1R2 and T1R3 and the presence of multiple binding sites on the sweet taste receptor [91–94]. Similar to other class C GPCRs, the N-terminal domain of T1R2 is responsible for binding to the sweeteners aspartame and neotame [91]. However, the human-specific residues of T1R3 (amino acids 536–545) within the cysteine-rich region, linking a large extracellular N-terminal domain to the transmembrane domain, are required for responsiveness to brazzein, a plant protein that tastes sweet to humans but not mice [92]. In addition, the C-terminal transmembrane domain of T1R3 is required for recognizing the other sweetener, cyclamate [91, 93]. On the other hand, the analysis of ligand interaction with the large extracellular N-terminal domains of T1Rs, expressed in *Escherichia coli* and purified, suggested that both the N-terminal domains of T1R2 and T1R3 bind sucrose and glucose with distinct affinities [95]. Although in the T1R2/T1R3 heteromer one subunit may modify the ligand sensitivity of the other subunit, the purified N-terminal domain of T1R3 potentially binds sucrose with higher affinity than does that of T1R2 [95]. The relationship appears to be reversed for glucose [95]. However, it remains elusive how the sugar occupation of binding sites on T1R2 and/or T1R3 in the heteromer contributes to the efficient activation of G protein. Since the T1R1/T1R3 heteromer is not activated by sugars, the T1R2/T1R3 heteromer may be activated by sugar binding to both T1R2 and T1R3, or by sugar binding to T1R3 associated with obligatory conformational changes of T1R2. Alternatively, the T1R2/T1R3 heteromer may be activated by sugar binding to T1R2, while being supported by intersubunit conformational coupling. G protein coupling is likely to require the transmembrane domain in the C-terminal half of T1R2 [91]. It is also of note that lactisole, a broadly acting sweet antagonist, suppresses the sweet taste of sugars, sweet-tasting proteins and artificial sweeteners by specifically binding to the C-terminal transmembrane domain of human T1R3 [91, 94]. Therefore, the further molecular dissection of lactisole action may help resolve an interesting aspect regarding how binding of a diverse array of sweeteners to multiple sites leads to the activation of G proteins through the common backbone dynamics of the receptor conformations.

Both T1Rs and T2Rs are partially coexpressed with the α-subunit of the G protein gustducin, a taste-specific signaling molecule [63, 82, 96]. Mice lacking α-gustducin show markedly reduced, but not completely abolished, behavioral and electrophysiological responses to bitter, sweet and umami compounds [97–99]. Thus, the gustducin heterotrimer plays substantial roles in bitter, sweet and umami transduction *in vivo*. Rod α-transducin ($\alpha_{\text{t-rod}}$) is also expressed in taste receptor cells at much lower levels than is α-gustducin [100, 101]. Behavioral and

electrophysiological data from mice lacking $\alpha_{t\text{-}rod}$ and/or α-gustducin revealed that $\alpha_{t\text{-}rod}$ is additionally involved in responses to umami compounds, but not to bitter and sweet compounds [99]. Interestingly, transgenic expression of a dominant-negative form of α-gustducin from the gustducin promoter has further diminished the residual bitter and sweet responsiveness of α-gustducin knockout mice [102]. The results suggest that other G protein α-subunits than α-gustducin, expressed in the α-gustducin lineage of taste cells, mediate a part of the sweet and bitter responses. Gαi2 that is reported to be expressed in taste receptor cells may be additionally involved in taste perception [98, 103]. Of the β- and γ-subunits of heteromeric gustducin, taste receptor cells express Gγ13 and Gβ3 [104, 105], which can activate phospholipase C, leading to the generation of inositol-1,4,5-trisphosphate (IP3) [104]. Recently, it was reported that the signal transduction molecules phospholipase Cβ2 (PLCβ2) and the TRPM5 ion channel are expressed in sweet, umami and bitter taste receptor cells [106–108]. TRPM5 forms ion channels permeable to monovalent cations but impermeable to Ca^{2+}, and the channel is directly gated by rapid increases in $[Ca^{2+}]_i$ [109–111]. Importantly, mice lacking either PLCβ2 or TRPM5 display diminished behavioral and nerve responses to sweet, umami and bitter taste stimuli, suggesting that both receptor families converge on a common signaling pathway in the cells (Fig. 1c) [112].

Thus, in bitter, sweet and umami taste transduction, binding of taste compounds to G-protein-coupled receptors appears to lead to dissociation of the heterotrimeric G protein. Then, $\beta\gamma$ subunits of the G protein activate PLCβ2, which hydrolyzes PIP2 into DAG and IP3. Subsequently, IP3 activates the type III IP3 receptor [107], leading to the release of Ca^{2+} from intracellular stores [113]. Rapid increases in $[Ca^{2+}]_i$ open basolaterally located TRPM5 channels, leading to the Na^+ influx and membrane depolarization which might be required for the neurotransmitter release. Although the GPCR-mediated bitter and sweet signals elicit changes in cAMP and cGMP levels via activation of α-gustducin and the other Gα molecules [114–118], which regulate opening or closing of several channels, those signaling pathways alone would not trigger nerve responses to bitter and sweet stimuli, but rather might modulate them.

Recent data demonstrated that increasing temperature between 15 and 35 °C activates TRPM5 in the presence of cytosolic Ca^{2+}, and enhances the gustatory nerve responses selectively to sweet compounds, but not to umami, bitter, sour and salty stimuli, in wild-type mice but not in TRPM5-deficient mice [119]. The mechanisms may underlie enhanced sweetness perception at high temperature [119]. However, it remains unclear how increasing temperature selectively enhances sweet perception but not bitter and umami perception, depending on TRPM5, although sweet, umami and bitter taste receptor cells use common signaling pathways involving PLCβ2 and TRPM5 for the generation of a taste response. Are circulating hormones such as leptin [120] involved in selective appearance of temperature dependence on sweet perception in taste receptor cells? Are the GPCR-mediated, sweet-selective signaling pathways such as cAMP-mediated regulation of K^+ and Ca^{2+} channels [1], or selectively high expressions of voltage-gated channels in sweet taste receptor cells involved in it? Electrophysiological analyses at a taste cell level may resolve the question. Of note, TRPM5 activity is stimulated by rapid increases in $[Ca^{2+}]_i$ and also regulated by $PI(4,5)P_2$: after Ca^{2+}-mediated activation, TRPM5 channels undergo rapid Ca^{2+}-dependent desensitization, which is partially reversed by PIP2 [110, 121]. Thus, either sweet-selective signaling pathways, selectively high expression of a certain channel or hormonal regulation might elicit the specific mode of changes in $[Ca^{2+}]_i$ and $PI(4,5)P_2$ in sweet taste receptor cells, compared with bitter or umami taste receptor cells.

Taste coding in the periphery

How is taste information encoded in the periphery? Recent discovery of T1Rs and T2Rs led to a clear view for the coding of sweet, umami and bitter taste in the periphery [72, 85, 112]. Most of the members of the T2R receptor family are co-expressed in the same subset of taste cells [63]. Thus, this cell type appears to recognize a diverse array of structurally distinct bitter compounds without a fine discriminatory power among different bitter compounds [72, 122]. Although T2Rs-positive cells and T1Rs-positive cells are localized in the same taste buds, T2Rs and T1Rs are not co-expressed in the same taste receptor cells [82]. Furthermore, there is no coexpression of T1R1 and T1R2 [76], suggesting that sweet, umami and bitter taste modalities are encoded separately by the activation of distinct cell types (Fig. 1c, Fig. 2a).

This view was further confirmed by genetic approaches. Mice lacking PLCβ2 have diminished behavioral and nerve responses to sweet, umami and bitter taste stimuli [112]. In PLCβ2-deficient mice, the PLCβ2 transgene expressed under the control of the mT2R5 promoter rescued the response to multiple bitter compounds, but not to sweet or umami taste [72, 112], supporting the notion that these modalities are recognized independently in distinct cell types. Importantly, the PLCβ2 rescue using the mT2R5 promoter recovered responses not only to cycloheximide, a ligand for mT2R5, but also multiple bitter compounds. The same rescue using the other two divergent T2Rs (mT2R19, and mT2R32) located in different chromosomal locations similarly recovered responses to multiple bitter compounds [72]. Therefore, these data

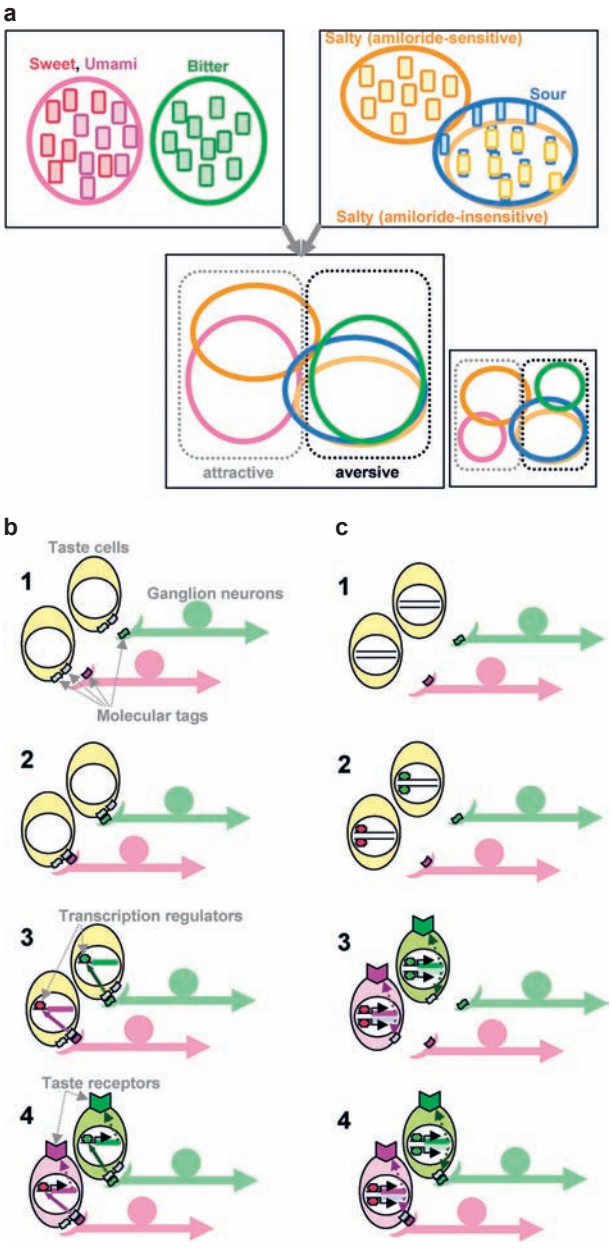

Figure 2. Taste coding in the periphery. (*a*) The proposed models of taste coding in the periphery. Separate populations of taste cells are uniquely tuned among sweet, umami and bitter taste (upper left), and among amiloride-sensitive salt taste and amiloride-insensitive salt taste (upper right). Although sweet cells are separated from umami cells, they are converged on one population in order to simplify the model. Two views are converged on one view (below left). The overlapping populations might vary among species (below right). (*b*, *c*) The schematic representation of the proposed models of taste receptor transcription and its nerve dependence. (*b*) The specific nerve fibers (ganglion neurons) contact common progenitor cells using molecular tags, activate a series of transcriptional regulators for the specific receptor to express it and thus differentiate the progenitor cells to specific taste receptor cells. (*c*) Independent of ganglion neurons, the progenitor cells differentiate to express the specific taste receptor and the molecular tag, controlled by interaction with surrounding cells, and thereafter contact and form a synapse with specific afferent fibers using specific molecular tags. A molecular tag is either same as or different from a taste receptor.

suggest that the T2R-expressing cells coexpress the multiple T2Rs, and are capable of responding to a broad array of bitter compounds. Furthermore, transgenic mice expressing a RASSL k-opioid receptor in T1R2-expressing sweet cells become selectively attracted to the synthetic opioid agonist spiradoline, which is a normally tasteless compound, implying that activation of sweet cells rather than the sweet receptor itself results in sweet perception [85]. In sharp contrast, transgenic mice expressing the same receptor in bitter cells substantially display aversion to spiradoline [72]. Thus, activation of a specific type of taste cells (sweet cells or bitter cells) and its line of neurons is dedicated to elicit a specific behavior (attractive or aversive). It is also suggested that aversive responses are independently elicited without requirement of a functioning attractive pathway.

Coding of sour and salty taste remains less clear. However, animals lacking sweet and bitter reception and perception still respond to sour and salty stimuli [112], revealing that sour and salty tastes are detected independently of bitter and sweet tastes. Are separate populations of cells uniquely tuned to these ionic tastes? Are salt and sour stimuli perceived in the same cells that mediate sweet, umami or bitter tastes without crosstalk in signaling pathways, while eliciting distinct temporal patterns of action potentials in taste receptor cells and afferent nerve fibers [123–125]? The identified view among taste coding of sweet, umami and bitter in the periphery may lead to the impression that the perception of five taste modalities results from the activation of five different cell types that transmit individual taste information along labeled lines to the brain. I do not think that taste coding simply fits that notion. Indeed, results from electrophysiological and imaging studies show that individual taste cells and individual gustatory neurons at several different levels are often activated by taste stimulation with two or more basic qualities [126–131]. Nevertheless, when compared among salt and sour taste, distinct populations of cells appear to be uniquely tuned to these ionic tastes [26, 27, 132, 133]. By using the selective sodium taste inhibitor amiloride, the single-unit recording of the chorda tympani nerve fibers demonstrated the existence of two types of nerve fibers transmitting salt taste information [24–27]. One type (N-type) receives input from taste receptor cells, which narrowly respond to NaCl and LiCl among salts, and the NaCl responses of which are strongly inhibited by amiloride. The other type (H-type) receives input from taste receptor cells that are responsive to various salts and also to acids, and are insensitive to amiloride [24–27]. These suggest that N-type neurons selectively innervate and form a synapse with amiloride-sensitive salt taste cells [26, 27]. Separately, H-type neurons selectively innervate a distinct population of cells that are amiloride-insensitive salt taste cells [26, 27]. Most of amiloride-insensitive salt taste cells may also perceive

sour taste, possibly because of either the usage of common signaling molecules or coexpression of distinct signaling molecules involved in the amiloride-insensitive salt and sour taste. However, the possibility that some of H-type neurons always and consistently innervate two distinct cell types of amiloride-insensitive salt taste cells and sour taste cells might not be excluded. Importantly, a substantial population of sour-responding cells may also be responsible for amiloride-insensitive salt taste, although some of them might be overlapping with amiloride-sensitive salt taste cells while employing the distinct molecule as an initiator for sour transduction. Of note, inputs from amiloride-sensitive and amiloride-insensitive taste cells appear to remain largely segregated in the solitary tract nuclei [133], suggesting an essential role of the distinct inputs in quality-coding among various salts. Thus, when considering salt and sour taste, selective activation of distinct populations of taste cells and their lines of neurons by NaCl (which activates both amiloride-sensitive and -insensitive cells), nonsodium salts (which activates amiloride-insensitive cells) and sour stimuli might lead to behavioral discrimination, largely dependent on their concentrations [27] (Fig. 2a).

Distinct populations of taste cells and afferent neurons are uniquely tuned to among sweet, umami and bitter taste, and among amiloride-sensitive salt taste and amiloride-insensitive salt taste (which is largely overlapping with sour taste). Convergence of the two views on one view might lead us to understand taste quality coding (Fig. 2a). Importantly, when we consider taste quality coding, it is necessary to precisely define input events eliciting individual taste transduction. In this manuscript, T1R2/T1R3-, T1R1/T1R3- and T2Rs-expressing cells are considered as the only initiators for sweet, umami and bitter taste, respectively. Although the definition might lead to side views of the fact, it might be necessary for fair comparison. Some taste compounds elicit the combined activity of cells tuned to different taste modalities. For example, quinine that is commonly applied as a bitter substance may activate not only T2Rs-expressing cells, but also the other taste receptor cells through the blockage of K^+ channels [134–136]. The effects may underlie differences in the chorda tympani versus glossopharyngeal nerve response ratio to quinine and cycloheximide that selectively activates T2Rs-expressing cells at a low concentration [137]. Bitter tastants such as caffeine and theophylline may activate taste receptor cells responsible for other taste modalities by modulating $[cGMP]_i$ through inhibition of phosphodiesterase, independent of a G-protein-mediated pathway [138]. Since those bitter compounds predominantly activate T2Rs-expressing cells over the other taste receptor cells, humans may taste them as bitter. However, when T2Rs-expressing cells are defined as the only initiators of bitter taste, the events triggered in other taste receptor cells must be considered as side effects, although the existence, quality and intensity of such side effects might be important for discrimination among various bitter compounds. Combination of electrophysiological recording from taste cells and single-cell reverse transcriptase polymerase chain reaction (RT-PCR) to identify the T2Rs expressed may help clarify whether bitter compounds selectively activate T2Rs-expressing cells or possess side effects. Monosodium glutamate may evoke both salty and umami taste because of its Na^+ content [85]. When T1R1/T1R3-expressing cells are defined as the only initiators of umami taste, the events triggered in salt taste receptor cells must be considered as side effects, although humans might taste umami for L-glutamate in the presence of some Na^+. Thus, to deduce umami responses in recording of taste responses to monosodium glutamate from taste cells and nerves, both activities of the amiloride-sensitive and -insensitive Na^+ channels need to be inhibited by using known blockers. In addition, many sweeteners exemplified by saccharin and acesulfame K are likely to activate T2Rs-expressing cells in addition to T1R2/T1R3-expressing sweet cells, accounting for the bitter aftertaste of saccharin [139]. KCl may activate not only amiloride-insensitive salt taste cells but also multiple taste receptor cells by eliciting membrane depolarization through various kinds of apically and basolaterally located K^+ channels. The potency of the side effects strictly depends on concentrations of compounds used, and also the experimental procedures. Under these considerations, we need to learn from the previous reports, which contain voluminous beautiful single-unit recordings of taste responses from both taste receptor cells and neurons, to deduce the view of taste quality coding. Even under these considerations, individual taste cells and individual gustatory neurons at several different levels are often responsive to stimuli representing more than one of the five taste qualities [126, 127, 140, 141]. However, sweet, umami and bitter taste modalities appear to be encoded separately by the activation of three different cell types [72, 85, 112]. Also, among amiloride-sensitive and -insensitive salt, and sour taste, activation of distinct populations of taste cells and afferent neurons is likely to transmit individual taste information to the brain [26, 27, 132, 133]. Nevertheless, previous reports clearly demonstrated that populations of individual sweet, umami and bitter cells are not completely separated from the populations of salt and sour cells [126, 127], although overlapping populations might vary among species [140, 142, 143]. Thus, subsets of amiloride-sensitive and -insensitive salt, and sour taste receptor cells seem to operate in the same cells that mediate sweet, umami or bitter tastes without crosstalk in signaling pathways between the modalities (Fig. 2a). Activation of distinct signaling molecules in salt and sour transduction from those in sweet, umami and bitter transduction may elicit the distinct mode of changes in $[Ca^{2+}]_i$ and the membrane

potential, resulting in the generation of distinct temporal patterns of action potentials in afferent nerve fibers. Even among sweet, umami and bitter taste cells which use common signaling pathways to trigger nerve responses, non-overlapping sets of signaling molecules may specifically modify the changes in $[Ca^{2+}]_i$ and the membrane potential in both cyclic nucleotide (cAMP and cGMP)-dependent and -independent fashion [1, 61]. As a result, the relevant temporal patterns of action potentials in afferent nerve fibers apparently convey information about the nature of taste stimuli [125], strictly dependent on the stimulus intensity. However, at present, it is difficult to prove their involvement in and their relative contribution to taste quality coding by showing that manipulation of the firing patterns in specific neurons affects the ability of taste discrimination and the appearance of taste-induced behavioral responses. Although temporal coding might be additionally involved, the activation of both specific lines of cells and specific populations of cells may substantially underlie taste quality coding. Accordingly, voluminous data revealed that neurons in ganglions and the solitary tract nuclei and even in the gustatory cortex are differentially sensitive to those taste compounds [7, 140–145]. Although neurons in the parabrachial nuclei and the gustatory cortex are also reported to elicit time-varying gustatory responses with more broadly tuned characteristics, their properties may be derived from interactions with other neurons within the same nucleus, and with other gustatory nuclei that provide either ascending or descending inputs [123, 124, 131, 146].

How is taste information processed in the central nervous system? Previous electrophysiological data elegantly provided information as to how gustatory information is processed and integrated by the specific nuclear relays in the brain [3, 7, 128, 131], implying that the gustatory neurons responding the best to any one among basic taste qualities are topographically organized [7]. However, the precise dimensions and internal organization for each taste modality remain unclear. To address the issue, we recently applied a genetic approach to delineate the neuronal circuitries of bitter and sweet taste by selectively expressing the fluorescently labeled transsynaptic tracer tWGA-DsRed in either bitter- or sweet/umami-responsive taste receptor cells in mice [147]. The plant lectin WGA is well known to function as an anterograde or a retrograde tracer. Thus, tWGA-DsRed, which originated from taste receptor cells and was transferred to the neurons in the solitary tract nuclei, may be further transferred either along their ascending pathways anterogradely, along neuronal circuitries within the same nuclei and to the medullary reticular formation, or to the neurons which provide the descending inputs to the solitary tract nuclei retrogradely [3, 148, 149]. However, the labeling density is influenced by the efficiencies of transport and degradation of the tracer. Their efficiencies appear to be governed by the anatomical and functional differences of the axonal and synaptic systems, which may vary among neuronal types. The neurons, which contained only a small amount of tracer and were not detected, might also relay taste information. Nevertheless, locations of the neurons containing a detectable amount of tWGA-DsRed revealed segregation of bitter and sweet inputs in the known gustatory area in the brain [147], implying that the ascending input pathways are more effectively labeled. Thus, the spatial distribution of tWGA-DsRed that was transferred from either bitter- or sweet/umami-responsive taste receptor cells suggested that the gustatory neurons, dispersed in the solitary tract nuclei, the parabrachial nuclei, the thalamic gustatory area and the gustatory cortex, may be organized with sweet/umami inputs rostral and with bitter inputs caudal, except for bitter inputs into the external lateral and external medial subdivisions of the parabrachial nuclei [147]. In the gustatory cortex, the dispersed areas of tWGA-DsRed-labeled neurons in two strains appeared to partly overlap along the anterior-posterior axis [147]. Electrophysiological characterization of tWGA-DsRed-labeled neurons under pharmacological and genetic manipulation of centrifugal modulation and the interactions with other neurons within the same nuclei is now of critical relevance for understanding the neuronal properties underlying taste discrimination, and thus for clarifying taste coding in the central nervous system.

Conclusion and perspectives

Among sweet, umami and bitter taste, the activation of three different cell types likely transmits individual taste information along labeled lines to the brain, leading to specific and contrastive behavioral responses: aversion for bitter, and attraction for sweet and umami. Also, among amiloride-sensitive and -insensitive salt, and sour taste, distinct populations of taste cells and their lines of neurons appear uniquely tuned. Among five taste modalities, populations of sweet, umami and bitter cells may partly overlap with populations of salt and sour cells. Thus, subsets of either amiloride-sensitive or -insensitive salt, or sour taste receptor cells also respond to sweet, umami or bitter stimuli without crosstalk in signaling pathways between the modalities, thus by the use of independent signaling molecules in taste receptor cells. The levels of functional expression of key signaling molecules for each taste signal transduction pathway may determine the taste sensitivity of receptor cells, which often respond the best to any one among basic taste qualities. Thus, this convergence of two patterns of line coding might lead to the notion that taste information is encoded by the activation of specific populations of cells.

Clarification of the molecular mechanisms underlying the transcriptional regulation of taste receptors or key down-

stream signaling molecules, and of their dependence on afferent nerve fibers which transmit specific taste information, might lead us to a clearer understanding of taste quality coding (Fig. 2b, c). One extreme possibility is that specific nerve fibers contact common progenitor cells, induce the expression of the specific receptor and thus cause the cells to become specific taste receptor cells (Fig. 2b). The other extreme is that independent of afferent nerve fibers, the progenitor cells differentiate to express specific taste receptors, for example, controlled by interaction with surrounding cells, and thereafter contact and form a synapse with specific afferent fibers (Fig. 2c). Precedent reports electrophysiologically addressed this issue between amiloride-sensitive and -insensitive salt taste cells and afferent fibers in combination with nerve regeneration [26, 27]. The further molecular dissection of the mechanisms which underlie the transcriptional regulation of taste receptors, solidly identified as initiators of signal transduction, and of their dependence on afferent nerve fibers may help define taste quality coding.

The sense of taste evokes contrastive behavioral responses. How is taste quality coding linked to behavioral output? This question is now addressed by electrophysiological recording of specific neurons that are innately tuned for attractive and aversive stimuli, and are linked to specific behavioral motor output [150, 151]. The genetic approach to visualizing the connections formed by the neurons which process individual taste information, in combination with electrophysiology, may be valuable for investigating the neuronal and molecular aspects underlying the linkage of taste inputs to contrastive behavioral motor outputs. To this end, because of the relative simplicity of the brain structure and the availability of genetic approaches, other organisms such as *Drosophila*, in which activation of selectively tuned taste receptor cells elicits either aversive or attractive behavioral responses, may provide an excellent model system and important information for understanding those aspects [152, 153]. On the other hand, salty and sour tastes are generally attractive and aversive modalities, respectively. However, those tastes also evoke the converse responses, dependent on their concentrations. Therefore, it is interesting to address how such a switching of attractive and aversive responses occurs, and whether it depends upon activation of specific lines or patterns, or upon changes in the precise firing pattern of action potentials.

Finally, mammals learn to reject an attractive tastant if this tastant is associated with subsequent visceral malaise [2]. It is also interesting to know the neuronal and molecular aspects underlying the taste learning. By visualizing the neuronal circuitries which originate from the specific neurons, and by evaluating changes in neuronal circuitries in combination with electrophysiological characterization and ablation of specific neurons, it will be understood whether or not the taste learning is associated with construction of new neuronal circuitries, and which neurons or which molecules play a key role in it. Thus, since the sense of taste evokes contrastive behavioral and emotional responses, the gustatory system may provide a simple model to deeply learn the general neuronal mechanisms which underlie cognition, behavioral and emotional responses, and learning.

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