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Sweet Successes

Minireview

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Mapping of the chromosomal location of genes essential for sweet and bitter taste and identification of the relevant G protein-coupled receptors reveals unanticipated complexity in taste signaling pathways. The distribution of sweet and bitter receptors suggests complete cellular segregation of these taste modalities. Sweet compounds may be distinguished through differential expression of sweet receptors. Novel heterologous expression systems to test bitter and sweet modalities now provide the tools necessary for understanding taste coding.

Long before we were old enough to appreciate the finer things in life, we were taught that tastes could be divided into a small number of distinct qualities. These sensations—bitter, sweet, sour, salty, and umami (monosodium glutamate)—have been explored with psychophysical methods for decades. The recent identification (Kitagawa et al., 2001; Matsunami et al., 2000; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001) and functional expression (Nelson et al., 2001) of receptors for sweet taste represent the culmination of efforts by multiple groups to combine data from genetic linkage mapping of sweet taste defects with the rapidly expanding genomic information available in the mouse. These studies, in conjunction with previous successes in identifying bitter taste receptors (Chandrashekar et al., 2000; Matsunami et al., 2000), have provided significant insights into the molecular basis of taste qualities and the coding of tastants at the biochemical, cellular, and electrophysiological level.

Early psychophysical and nerve fiber recording studies suggested a simply organized taste coding system where a stimulus is categorized into a class (sweet, for example) but its taste quality is not further distinguished within that class. This model suggested limited receptor diversity would be needed for taste coding. Recording responses within a mammalian taste bud has been complicated by the limited ability to access individual taste cells and deliver appropriate stimuli. The application of improved Ca^{2+} imaging methods to studies of bitter taste has recently raised doubts regarding the earlier simple model for taste discrimination. Single bitter-responsive cells within a taste bud display distinct activation patterns to a range of stimuli (Caicedo and Roper, 2001), suggesting that the bitter taste modality could be separated into subclasses. However, the functional importance of this variation for taste discrimination within a given modality remains unclear.

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In parallel with the physiological characterization of taste, classical genetics led to the identification of a few loci in mice displaying simple mendelian inheritance that defined thresholds for detection of sweet tastants (Fuller, 1974) and aversive responses to bitter compounds (Lush, 1984; Lush and Holland, 1988). These studies suggested a small number of taste receptors were present in these animals and encoded at these sites. Although the small numbers of taste cells initially hampered molecular characterization of receptors, single-cell library technology has accelerated the elucidation of taste transduction components. Over the last year, several groups have exploited these genetic and molecular biological leads to identify candidate taste receptors.

The Sac *Locus and Sweet Taste Receptors*

Humans and rodents perceive a broadly similar range of compounds as sweet, and this conserved taste perception extends to some artificial sweeteners including saccharin. Five-fold differences in detection thresholds for this sweet compound among inbred mouse strains allowed the identification of a region on chromosome 4, the *Sac* locus, responsible for the phenotypic variation (Capeless and Whitney, 1995; Fuller, 1974). *Sac* tasters and nontasters also share similar variation in detection thresholds for other naturally sweet compounds. These observations led to the conclusion that a sweet taste receptor or other key component in this transduction pathway was encoded at the *Sac* locus.

Two G protein-coupled receptors (GPCRs) with specific expression in taste cells, first identified through differential cDNA library screening, were mapped in the vicinity of *Sac* (Hoon et al., 1999). However, these genes were eliminated as potential candidate genes responsible for the *Sac* phenotype by high-resolution genetic linkage analysis (Li et al., 2001). The recent availability of mouse and human genome sequences fueled a new search for GPCR genes near the *Sac* locus. A new candidate taste receptor, T1R3, was expressed in taste buds and displayed homology to the adjacent GPCRs previously mapped outside of the *Sac* critical interval (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Nelson et al., 2001; Sainz et al., 2001). Subsequent analysis of sequence-specific differences in T1R3 between Sac taster and nontaster strains revealed amino acid substitutions that provide highly suggestive albeit not definitive evidence that this gene encodes a sweet taste receptor. The identification of this putative taste receptor highlights the rapid advances made possible with availability of complete sequences for mammalian genomes.

A series of experiments utilizing the T1R3 gene and protein provide compelling evidence that this protein is critical for sweet taste (Nelson et al., 2001). First, expression of a native promoter-driven T1R3 gene derived from a taster strain in a nontaster transgenic mouse led to phenotypic rescue of the *Sac* deficit. This observation confirms that T1R3 is necessary for sweet taste transduction but does not address sufficiency. Secondly, heterologous expression of T1R3 GPCR, in combination with the other GPCRs adjacent to the Sac locus

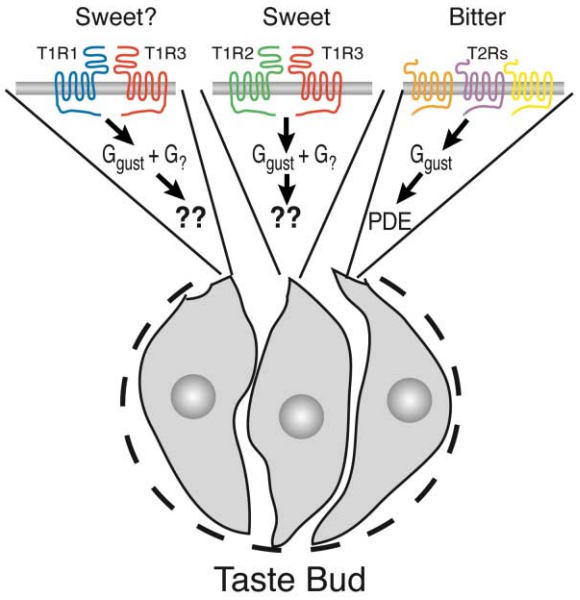

Figure 1. Schematic Diagram of Receptor Distribution and Signaling Pathways in the Sensory Cells of a Taste Bud

The distribution of the T1R3 GPCR and its association with T1R1 and T1R2 receptors in sweet-responsive cells is inferred from in situ hybridization. The presence of multiple T2R receptors within individual bitter-responsive cells expands the repertoire of ligands detected by each cell. The putative role of G_{gust} and additional G proteins in activation of PDE and unidentified effectors for sweet taste is suggested by G_{gust} knockout mice.

results in a functional sweet taste receptor (see below). Together, these experiments confirm that T1R3 is an essential component of sweet taste transduction.

Receptor Expression

The presence of three sequence-related receptors (T1Rs) in proximity to the *Sac* locus suggested that each might be responsible for detecting a subset of the broad range of sweet compounds detected by mammals. Olfactory receptors display a similar chromosomal organization, where related family members detect distinct odorants. In the olfactory system, expression of receptors in nonoverlapping patterns is likely to be critical for discrimination among odorants. In contrast, the T1R taste genes were found to be expressed in a more complex, partially overlapping pattern (Figure 1; Nelson et al., 2001). Specifically, the T1R3-positive cells appear to also express one or the other of the two other members of the family (T1R1 or T1R2).

The overlapping expression of multiple T1R family members could contribute to a broadened response profile for individual sweet-responsive cells or reflect a requirement for multiple subunits in the formation of a functional receptor. The sweet tastant-mediated activation of second messengers in a heterologous expression system requires coexpression of T1R2 and T1R3; the expression of individual T1Rs failed to elicit a response. This observation suggests that the functional sweet receptor is a heteromultimer comprised of these subunit combinations.

Multimerization of receptors has not previously been described for chemoreceptors, although chaperones for GPCRs that also modulate ligand selectivity have already been described in other systems (McLatchie et al., 1998). Assembly of the T1R GPCRs into multimers might similarly afford increased ligand sensitivity. Alternatively, T1R3 could serve as a common chaperone essential for proper cellular localization of T1R1 and T1R2. Altered function of T1R3 in nontaster strains might for this reason result in reduced responses in all sweet taste-responsive cells. These novel findings in the taste system may stimulate a search for similar heteromerizing receptors in the olfactory system, for cases where individual odorant receptors, like individual T1Rs, fail to functionally express in heterologous systems.

Genetic Diversity

In contrast to neurotransmitter and peptide hormone GPCRs, which are highly conserved, mammalian chemosensory receptor genes display considerable divergence between species. Human and mouse olfactory receptor paralogs display on average just 58% nucleotide sequence identity (Lane et al., 2001). Likewise, members of the multigene T2R taste receptor family display no more than 70% sequence identity between species. This trend could reflect the different sensory environments driving evolution of the two species or rapid drift arising from the existence and organization of multigene families. The existence of comparable diversity in the T1R family, consisting of only three members, suggests that the former explanation is more likely.

In behavioral experiments, rodents fail to detect several natural and artificial ligands that humans perceive as sweet. While it is obvious that the odor environment for the two species is distinctly different, the variety of sweet tastants (sugars) in the diet might be thought to be quite similar. However, many of the most potent natural sweet tastants are plant derived and exist in unique ecological niches where they serve as attractants rather than as a caloric source. Consequently, humans may have lost the ability to detect some of the novel sweet substances that rodents can still enjoy. Similarly, many intensely sweet artificial compounds are capable of activating human sweet taste receptors but display modest effects on the divergent rodent proteins (Danilova et al., 1998).

Taste Coding

A single taste bud, consisting of dozens of sensory cells, has the ability to respond to multiple taste modalities. The identification of receptors for different classes of tastants is a valuable tool to examine whether the sensing of each modality is strictly segregated in nonoverlapping cells. The T1Rs and T2Rs, responsible for detecting some sweet and bitter compounds, respectively, are clearly expressed in distinct subsets of neurons within the same taste bud (Nelson et al., 2001). A taste-specific G_{α} subunit, gustducin, previously shown biochemically to mediate bitter taste transduction, is present in all T2R-positive cells. A genetic disruption of gustducin displayed a marked defect in bitter sensitivity as well as an unexpected reduced sensitivity to sweet tastants (Ruiz-Avila et al., 2001; Wong et al., 1996). The presence of gustducin in at least some T1R-positive cells (Adler et al., 2000; Max et al., 2001) may explain the behavioral deficit. Interestingly, gustducin null mice are not completely unresponsive to bitter and sweet compounds, suggesting the presence of additional signaling path-

ways (Wong et al., 1996). These observations could be interpreted to suggest overlapping transduction pathways, additional receptor families, or imperfect restriction of expression for transduction components. The contribution of individual α subunits in coupling to each receptor family remains unclear, but a rigorous examination of the expression patterns at the cellular level will hopefully clarify the situation.

The apparent segregation of taste modalities to independent cells does not address whether related receptors are utilized to distinguish among tastants within a given modality. It is possible that multiple receptors for a given modality are required to detect the broad range of taste compounds. Molecular evidence suggests that in bitter taste the expression of multiple T2R genes in each cell that funnel into a common second messenger pathway serves to create a more efficient bitter detector. The organism may only perceive bitterness (classically associated with toxic substances) rather than the precise identity of the compound. However, electrophysiological studies of taste cells have suggested some ability to distinguish among bitter compounds, although the significance to the organism remains unknown (Caicedo and Roper, 2001). The detection of sweet compounds could follow a similar logic where it is sufficient to simply detect caloric-rich food and unnecessary to distinguish among sweet compounds. The presence of T1R1 and T1R2 in nonoverlapping cells and the failure of T1R2/T1R3 receptors to respond to several sugars (Nelson et al., 2001) leaves open the possibility of perceptual discrimination between sweet tastes.

Structural Basis of Ligand Recognition

The ligands for sweet taste receptors possess several intriguing properties. For instance, the detection threshold for sugars is roughly 0.1 M, more than five orders of magnitude greater than observed for bitter compounds. Sugars could activate cognate receptors by binding at relatively low affinity to specific recognition sites in the protein. Alternatively, sweet receptors could be tuned to alter their conformation in response to more general alterations in the solvent environment. These changes, induced by hydroxyl-rich sugars present at high osmolarity, would be analogous to the ionic strength-dependent association of protein subunits in other systems. The Ca^{2+}-sensing receptor, a structurally analogous GPCR, is activated at $K_{act} \sim 3$ mM through multiple Ca^{2+} binding EF hand motifs present in its large N-terminal domain (Brown et al., 1993). The presence of a large, conserved N-terminal extension among T1Rs is consistent with sugars acting specifically and cooperatively through this domain. Potent artificial sweeteners are chemically unrelated to sugars and might act at sites distinct from those responsible for binding natural compounds. Interestingly, the T2Rs lack long N-terminal extensions and are structurally more related to the odorant receptors, which bind ligands within the transmembrane segments. The availability of a system for functional expression of taste receptors (Nelson et al., 2001) should permit direct identification of residues that mediate ligand recognition.

The recent progress in elucidating the molecular mechanisms of sweet and bitter taste is a template for a more comprehensive understanding of all taste modalities. The application of genomic and genetic methodologies to identify candidate genes in each of the other taste classes is leading to similar rapid progress. An understanding of taste sensation, from the biochemistry and electrophysiology of the sensory cell to the development of a perception, may be achieved in the not too distant future.

Selected Reading

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J., and Zuker, C.S. (2000). Cell *100*, 693–702.

Brown, E.M., Gamba, G., Riccardi, D., Lombardi, M., Butters, R., Kifor, O., Sun, A., Hediger, M.A., Lytton, J., and Hebert, S.C. (1993). Nature *366*, 575–580.

Caicedo, A., and Roper, S.D. (2001). Science *291*, 1557–1560.

Capeless, C.G., and Whitney, G. (1995). Chem. Senses *20*, 291–298.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S., and Ryba, N.J. (2000). Cell *100*, 703–711.

Danilova, V., Hellekant, G., Tinti, J.M., and Nofre, C. (1998). J. Neurophysiol. *80*, 2102–2112.

Fuller, J.L. (1974). J. Hered. *65*, 33–36.

Hoon, M.A., Adler, E., Lindemeier, J., Battey, J.F., Ryba, N.J., and Zuker, C.S. (1999). Cell *96*, 541–551.

Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y., and Hino, A. (2001). Biochem. Biophys. Res. Commun. *283*, 236–242.

Lane, R.P., Cutforth, T., Young, J., Athanasiou, M., Friedman, C., Rowen, L., Evans, G., Axel, R., Hood, L., and Trask, B.J. (2001). Proc. Natl. Acad. Sci. USA *98*, 7390–7395.

Li, X., Inoue, M., Reed, D.R., Huque, T., Puchalski, R.B., Tordoff, M.G., Ninomiya, Y., Beauchamp, G.K., and Bachmanov, A.A. (2001). Mamm. Genome *12*, 13–16.

Lush, I.E. (1984). Genet. Res. *44*, 151–160.

Lush, I.E., and Holland, G. (1988). Genet. Res. *52*, 207–212.

Matsunami, H., Montmayeur, J.P., and Buck, L.B. (2000). Nature *404*, 601–604.

Max, M., Shanker, Y.G., Huang, L., Rong, M., Liu, Z., Campagne, F., Weinstein, H., Damak, S., and Margolskee, R.F. (2001). Nat. Genet. *28*, 58–63.

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G., and Foord, S.M. (1998). Nature *393*, 333–339.

Montmayeur, J.P., Liberles, S.D., Matsunami, H., and Buck, L.B. (2001). Nat. Neurosci. *4*, 492–498.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J.P., and Zuker, C.S. (2001). Cell *106*, 381–390.

Ruiz-Avila, L., Wong, G.T., Damak, S., and Margolskee, R.F. (2001). Proc. Natl. Acad. Sci. USA *98*, 8868–8873.

Sainz, E., Korley, J.N., Battey, J.F., and Sullivan, S.L. (2001). J. Neurochem. *77*, 896–903.

Wong, G.T., Ruiz-Avila, L., Ming, D., Gannon, K.S., and Margolskee, R.F. (1996). Cold Spring Harb. Symp. Quant. Biol. *61*, 173–184.