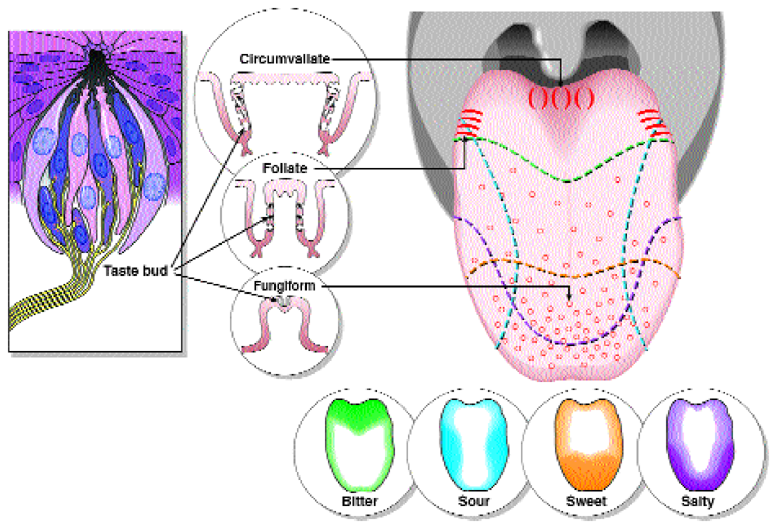
A MATTER OF TASTE

Candidates for Taste Receptors Identified



A map showing the location on the tongue of different kinds of taste buds and the regions of the tongue that respond to four types of taste.

CHARLES ZUKER/HHMI AT THE UNIVERSITY OF CALIFORNIA, SAN DIEGO

In a world of lime-laced tortilla chips, hoppy beers and countless flavors of ice cream, our sense of taste might seem to exist purely for pleasure. But the ability to distinguish bitter from sweet does far more than help us choose between light and dark chocolates. Sweetness is a signal that what we're eating will give us energy, while bitterness can warn that we may be about to swallow something toxic. In our primordial past—long before nutritional labeling—heeding such cues was essential to survival, as it still is for animals living in the wild.

Important as taste may be, its molecular underpinnings have remained a mystery. A team led by Howard Hughes Medical Institute investigator Charles Zuker, however, has taken a step toward solving part of that mystery. In collaboration with Mark Hoon and Nicholas Ryba of the National Institute of Dental and Craniofacial Research, Zuker and colleagues Elliot Adler and Jurgen Lindemeier of the University of California, San Diego have isolated genes encoding two proteins that may function as taste receptors. They published their findings in the February 19, 1999, issue of the journal *Cell*.

The researchers used the latest DNA cloning and screening techniques to generate and sift through libraries of rat genes. From these libraries, they identified a large number of genes that were expressed solely or primarily in taste buds. Two such genes, dubbed *TR1* and *TR2*, were especially promising candidates, says Zuker, because they resembled genes that encode other types of sensory receptors.

To be good receptor candidates, though, they needed to do more than simply resemble other receptors—they also had to be expressed in the right places. In mammals, taste receptor cells are organized into the onion-shaped clusters called taste buds. Taste buds reside in papillae, tiny protuberances that cover the upper surface and sides of the tongue. Different types of papillae are found on different parts of the tongue. At the very back of the tongue are circumvallate papillae, which seem particularly sensitive to bitterness. Along the sides of the back of the tongue are foliate papillae, sensitive to both sourness and bitterness. Fungiform papillae, located at the front of the tongue, are most sensitive to salty, sour and sweet compounds.

If TR1 and TR2 truly function in taste discrimination, their distribution should match in some way the distribution of different types of papillae, the researchers reasoned. Indeed, the team found that TR1 is expressed in nearly all fungiform taste buds, but is rare in taste buds of the bitter-sensitive circumvallate papillae. TR2 has the opposite distribution—it is rarely expressed in fungiform papillae but is expressed in all circumvallate papillae. Both TR1 and TR2 are expressed in foliate taste buds, but usually not in the same cell of a given taste bud.

That was encouraging, but still not enough to certify TR1 and TR2 as taste receptors. The next critical step was to find out exactly where their proteins are found in the receptor cells that make up taste buds.

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thing like the lobbies of busy office buildings: They receive stimuli from the outside world and then translate those stimuli into signals, which are sent on to the brain for interpretation. In a taste receptor cell, this bustling lobby is the taste pore. It is here, researchers believe, that molecules of sweet, sour, salty and bitter stuff link up with receptors, and the real work of taste recognition begins.

Using the office building analogy, taste receptors are like the security guards who ask visitors for identification and then send them to the appropriate floor. If TR1 and TR2 are bona fide taste receptors, you would expect to find them stationed at their

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Graduate students Yifeng Zhang (left) and Grace Zhao (right) discuss ongoing experiments with HHMI investigator Charles Zuker.

posts in the taste pores. Using antibody labeling techniques that can pinpoint the location of specific molecules, that's just what Zuker and his colleagues found. TR1 turned up only in the taste pore region of foliate and fungiform taste buds; TR2 was in the taste pores of circumvallate and foliate taste buds.

Zuker cautions that the evidence implicating TR1 and TR2 is, so far, only "guilt by association." The two proteins resemble other receptors, and they're found where you'd expect receptors to be, but more experiments are needed to show that they actually do function as taste receptors. One key experiment would be to show that they recognize, bind and are activated by molecules bearing the appropriate tastes. Another important study would be to generate "knockout" mice that lack TR1 or TR2 and see if these mice are incapable of making certain taste distinctions. Both types of studies are under way in the Zuker and Ryba labs.

Even before all the details are worked out, Zuker says that researchers can use TR1 and TR2 to begin probing other mysteries of taste. One of the biggest mysteries is how information received by cells in the tongue is transmitted to the brain. "Do you have, for example, coded lines of information, where there's a sweet line, a bitter line, a sour line, and a salty line? Or do you have mixed lines?" Zuker asks. And if the lines are mixed, how does the brain sort out signals so it knows whether you've just tasted lemon meringue or chocolate cream pie?

Because TR1 and TR2 are expressed in some cells and not others, scientists can use them to mark and study particular cells. "We can ask, for example, if all the cells that express one of these molecules are sending information via the same fibers to the brain, or are mixed with other cells that are different," says Zuker. "By doing this type of experiment, you can immediately begin to answer whether you have individual lines or mixed lines."

Probing differences among cells also could help researchers find out just how many taste receptors there are. After an exhaustive search, Zuker's and Ryba's group turned up only two, but that doesn't mean there aren't more. "There are zillions of sweet substances, and there are zillions of bitter substances that are all very different from one another," observes Catherine Dulac, an HHMI investigator at Harvard University who wrote about the Zuker team's findings in the February 1999 issue of *Neuron*. Carbohydrates, amino acids, proteins, inorganic salts and a variety of artificial sweeteners all can taste sweet, Dulac points out. "It would be difficult to conceive that only two receptors could accommodate this huge variety."

Zuker agrees, but thinks it's just as unlikely that thousands of different taste receptors will be found. "There is no doubt in my mind that sweet receptors evolved with the sole purpose of identifying highly caloric food sources and bitter receptors to warn the organism of noxious stimuli, essentially saying, 'This is bad news, get the hell away from it!'" So what's important to survival, he says, is the ability to distinguish between bitter and sweet, not to make fine distinctions within each taste category.

From the perspective of a foraging mammal, "I just want to know that something is bad news; I don't need to discriminate it from other bad news," explains Zuker. "So I strongly believe that the repertoire of taste receptors is likely to be way smaller than the olfactory universe, where you have a thousand receptors. But at the other extreme, I think one is too few." And without doing the research, he adds, there's really no way to predict how many taste receptors may turn up.

Toying with taste is a bit of a switch for Zuker, who is best known for his work on the visual systems of flies. "I'm a fly-eye kind of guy," he jokes. But his main interest is not so much in flies as in using their eyes as tools to understand sensory signaling. About three years ago, his research group decided to study a different sense—taste—and compare it with others, such as vision. They started out working with flies, but soon realized that isolating individual taste receptor cells from such tiny creatures was going to be mighty tricky.

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"So that's when we made a leap and said, 'Let's try something bigger,"' Zuker recalls. "We tried cows, which was a hideous experience. Do you know how big a cow's tongue is? We're talking three and a half feet here!" Rats seemed a good compromise between the ridiculously tiny and the hideously huge.

"Right about the time that we started our rat work, we discovered the efforts that Nick Ryba and Mark Hoon were involved in at the National Institutes of Health, and we started a most wonderful, productive, collaborative joint effort," says Zuker.

From fly eyes to rat tongues, with a detour through the mouth of a cow—where will all this lead? Fittingly enough, it could be somewhere delicious. Understanding the molecular basis of sweet sensitivity could help researchers design more potent sweeteners for our sodas and snacks. And instead of simply masking unpleasant tastes—in medicines, for example—they might find ways of blocking bitterness altogether.

There may even be help for people whose problem is not blocking out awful tastes, but tasting anything at all. "As people get older," notes Zuker, "their enjoyment of food is often dramatically decreased"—probably because of diminished taste perception. By identifying and studying taste receptors and taste signaling pathways, researchers may be able to find molecules that act as modulators, enhancing the cell's capabilities. ■

New Tools May Help Demystify Taste

For a sense with such ancient evolutionary origins and such tantalizing appeal in everyday life, taste is poorly understood. "There are some very basic issues about taste that are completely unknown," says molecular biologist and HHMI investigator Charles Zuker. For instance, he says, "we know that we can taste these five different modalities—sweet, sour, bitter, salty and umami [the flavor of monosodium glutamate]. But is it true, as some people claim, that different parts of the tongue are preferentially sensitive to different tastes, or is that complete nonsense? We really don't know to what degree that is real."

Another open question, says Zuker, is "whether your tongue is the same as my tongue." If you have a "sweet tooth," is it because your sweet-sensitive receptors are somehow different in number, sensitivity, or arrangement from those of someone whose desk drawer isn't stuffed with Snickers? It's unclear, too, how cells in the tongue convert sensory information into signals and how those signals are transmitted to the brain.

"From the very basic to the very esoteric, there are a lot of voids which we know could become tractable problems with the right molecular tools," says Zuker.

Molecular tools have made all the difference in the field of mammalian olfaction, in which HHMI investigators Linda Buck and Richard Axel identified a family of about 1,000 receptors in 1991, sparking an explosion of new information about the sense of smell. Buck, then a postdoctoral fellow in Axel's Columbia University lab, continues to study olfaction as an HHMI investigator at Harvard University.

The quest for taste receptors has come along a little more slowly, in part because taste researchers have placed less emphasis on molecular techniques, but also because taste receptor cells are relatively sparse and difficult to isolate, says Zuker. Thanks to advances in understanding olfaction, however, taste researchers haven't had to start completely from scratch. Axel and Catherine Dulac, an HHMI investigator at Harvard University, taught Zuker's team the technique of single-cell polymerase chain reaction (PCR), a novel cloning strategy that in 1995 allowed Axel and Dulac to make a key discovery about receptors for pheromones, powerful scents released from animals that trigger specific behavioral and hormonal responses. This strategy, combined with subtractive screens pioneered by Mark Hoon and Nicholas Ryba at the National Institutes of Health, allowed Zuker's and Ryba's team to isolate a collection of taste-specific genes, including *TR1* and *TR2*.

Single-cell PCR, explains Dulac, makes it possible to investigate differences among individual cells. This is important in olfactory research because each sensory neuron is slightly different from its neighbor due to differences in receptor expression. And because the expression of taste receptors likewise differs from cell to cell, studying individual differences is a key to understanding how the whole taste system works.