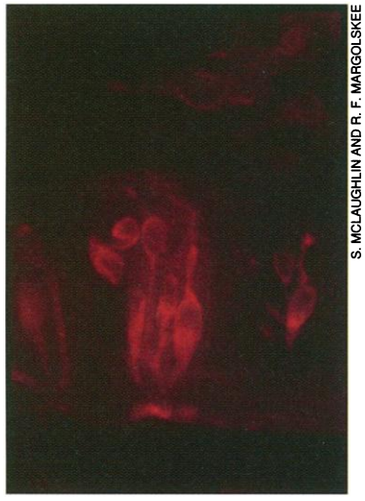
MEETING BRIEFS

Signaling Inside Neurons Takes Some New Twists

At Cold Spring Harbor Laboratory's annual Symposium on Quantitative Biology, devoted this year to neuroscience, neurobiologists basked in Long Island sunshine while discussing their recent work. Among the freshest new results were two that add surprising new twists to the stories of how certain neurons respond to incoming signals.

The Bittersweet Truth About Gustducin

Bitter and sweet are polar opposites in the world of taste. Humans and other animals generally avoid foods that taste bitter, an indication that they might be toxic, but can't get enough of those that taste sweet. Sweet and bitter even have opposing effects on the internal signaling pathways of the taste neurons in the tongue. Sugars boost levels of an intracellular signaling molecule called cyclic AMP in sweet-responsive cells, while bitter compounds, among other effects, lower cAMP levels in bitter-sensing cells.



S. MCLAUGHLIN AND R. F. MARGOLSKEE

Switch hitter. Gustducin, stained red in these taste cells, transmits sweet, as well as sour, taste signals.

That disparity suggested that different molecules transmit the sweet and bitter taste messages in the cells. But at Cold Spring Harbor, taste researcher Robert Margolskee of Mount Sinai School of Medicine in New York City reported that both messages are transmitted by the same protein, called gustducin. That opposing effects can somehow be produced by one protein comes as "a surprise," says taste physiologist Sue Kinnamon, of Colorado State University in Fort Collins.

The current finding is an outgrowth of work done in 1992, in which Margolskee, then at the Roche Institute of Molecular Biology in Nutley, New Jersey, and his co-workers cloned the gene for a taste bud–specific protein they called α-gustducin. They soon suspected that they had found a crucial relay protein for the taste cells. They knew that the receptors for sweet and bitter compounds send their signals to the cell interior via G proteins, a class of transducer proteins that bind the molecule GTP. And because the sequence of α-gustducin is remarkably similar to that of α-transducin, the G protein that transduces the light signal in the cells of the retina, the Margolskee team thought that it had a likely G protein for taste cells.

Or at least for one signaling pathway in those cells, namely the one for bitter taste. Transducin, α-gustducin's light-sensing counterpart, turns on an enzyme called phosphodiesterase (PDE), which lowers levels of cAMP. And it was well known that bitter compounds activate PDE in taste cells.

To see whether α-gustducin is in fact essential for detecting bitter sensations, postdoc Gwen Wong of the Margolskee group created a line of mice in which the gene for the protein was knocked out. Normal mice avoid water laced with quinine or a very bitter chemical called denatonium, but the knockout mice didn't seem to notice the bitter compounds, although they had normal responses to salt and sour, tastes that don't signal through G proteins.

The researchers were taken aback by another effect of the knockout, however. The mice showed no preference for sweetened water, although, Margolskee says, "wild-type mice love sugar and artificial sweetener," and will always choose sweetened water over plain. Unexpected as that finding was, the Margolskee team further nailed down α-gustducin's parallel involvement in both sweet and bitter tastes by showing that mutations that block α-gustducin's ability to interact with taste-receptor molecules on the cell surface interfere with an animal's sense of sweet as well as bitter. That, says Kinnamon, confirms that α-gustducin receives a signal directly from the sweet-receptor protein at the cell surface, meaning it is central to the sweet-sensation signaling pathway.

But how does α-gustducin translate the signal it receives into opposite effects for sweet versus bitter? Given gustducin's similarity to transducin, which interacts directly with PDE, it may be that gustducin gives opposite tweaks to PDE, destroying cAMP by turning up PDE activity in bitter-responsive taste cells and shutting down the enzyme—thus raising cAMP levels—in sweet-sensing cells. "That would be really cool if that were the case," Kinnamon muses. "That would be one way you could have a molecule that serves two very different functions."

NGF Shows Killer Instinct

In the drama of nerve cell life and death, nerve growth factor (NGF) is known as a good guy. Years of work have shown that it saves some populations of developing and adult neurons from committing suicide. But at the symposium, Yves-Alain Barde of the Max Planck Institute for Psychiatry in Martinsried, Germany, reported that NGF has a dark side: In some cases it kills baby neurons by binding to a receptor protein called p75.

This is less evil than it sounds—the neurons must die as part of normal development. But it does suggest a new view of neuron death, with NGF serving in some cases as killer, and p75 as its instrument. "It's very nice work," says Story Landis, of the National Institute of Neurological Disorders and Stroke. "Yves's data suggest that NGF ... can initiate cell death." But how it does the deed is a matter of dispute.

The dispute centers on the role of p75. Although it was identified as an NGF receptor in the early 1980s, researchers could never prove that p75 actually transmits NGF's signal inside neurons. Then in 1991, p75 was bumped from the limelight by the discovery of a second NGF receptor, called TrkA, which binds NGF more tightly than p75 does and seems to be the key receptor for mediating NGF's cell-saving effects. Subsequent studies suggested some rather humble auxiliary roles for p75; for example, it binds not only NGF but other neurotrophins as well, and in some cases it seems to improve the neurotrophins' efficiency by collecting them and handing them off to their respective receptors, all members of the Trk receptor family.

It wasn't long, though, until p75 was back in the limelight for a new reason. In the early 1990s biologists found a family of receptor proteins, including the tumor necrosis factor receptor (TNFR), that cause cells to commit suicide in situations ranging from immune-cell attack to the routine pruning of extra cells during development. The

amino acid sequences of these receptors resemble that of p75, suggesting that p75 belongs to the same family.

Evidence that p75 can kill cells was quick to follow. In 1993, Dale Bredesen's group at the University of California, Los Angeles, reported that naked p75, unbound to NGF, kills cells, and that NGF blunts the killing. Although that was consistent with NGF's well-known role as cell savior, the idea that bare p75 kills ran counter to the behavior of other TNFR family members, which kill only when bound to their activating molecule, says Bredesen, now at the Burnham Institute in La Jolla, California.

Indeed, the idea of p75 as a killing molecule was not widely accepted. For example, in 1994 when Mark Bothwell's group at the University of Washington, Seattle, found that NGF binding to p75 causes cell death in a population of brain neurons, they did not interpret this as direct killing by p75. They proposed instead that NGF binding to p75 was preventing p75 from carrying out its auxiliary role of facilitating the binding of other neurotrophins to their Trk receptors, and as a result, the cells were losing a life-sustaining signal.

Barde, however, decided to test the possibility that NGF might directly instigate p75 to kill cells. He thought NGF might do this very early in development, a time when there is a lot of neuron death that has not been very well studied. Barde's team chose to study retinal neurons in very young chick embryos, which contain p75 but not TrkA. Normally, half of those neurons die in a programmed mass suicide that eliminates an excess of nerve cells during the fourth day of embryonic development.

But when Barde's group injected chick embryos with a monoclonal antibody to NGF that blocks its binding to p75, 80% of the neurons that would have died were saved. Further evidence of NGF's role in the neurons' death came when the Barde group found that the neurons could also be saved by antibodies that block NGF by binding to p75. "Early in development, cell death is triggered by the ligand [NGF] known to do the opposite later," Barde concludes.

Bredesen notes that because the retinal neurons Barde was studying, while lacking TrkA, still have Trk receptors for other neurotrophins, it's possible that NGF is acting indirectly, as Bothwell had hypothesized. And both Bothwell's and Barde's observations remain in inexplicable conflict with Bredesen's. "It is a hot field," says Bothwell, "but it is not really focused into a clear picture yet." As that picture begins to sharpen up, p75 and NGF are visible as central figures, even though exactly what they are doing remains a blur.

–Marcia Barinaga

STRUCTURAL BIOLOGY

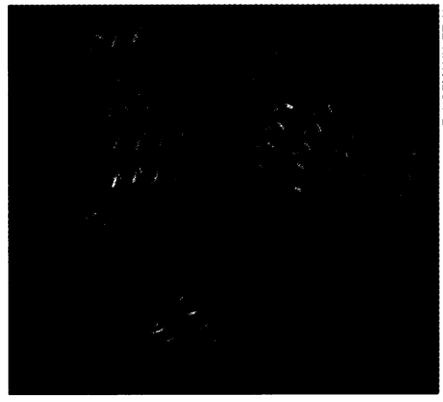
Form Follows Function When Plants Harvest Light

Anyone who has taken even an introductory biology class knows about the remarkable photosynthetic abilities of green plants. They can make complex organic molecules like sugars and starches from simple compounds like water and carbon dioxide, powered only by the energy they capture from sunlight. Far less well known, however, is a host of other photosynthetic organisms, including numerous bacteria and single-celled algae, such as the dinoflagellates that bloom periodically in poisonous "red tides." Obscure though they are, some of these organisms are masters of photosynthesis, harvesting wavelengths that green plants miss or converting light into stored energy with even greater efficiency. On page 1788, a team led by crystallographer Wolfram Welte of the University of Konstanz, Germany, and plant biologist Roger Hiller of Macquarie University in New South Wales, Australia, reports new findings that help explain why one of these organisms, the dinoflagellate *Amphidinium carterae*, is such an effective photosynthesizer.

From fluorescence studies, researchers already knew that the organism can capture light energy and transfer it with nearly 100% efficiency to the biochemical machinery that begins the job of converting it into chemical energy. Now, Welte, Hiller, and their colleagues have determined the structure, to a resolution of 2 angstroms, of one of *A. carterae*'s two light-harvesting "antennas." This is the peridinin-chlorophyll-protein (PCP), which is so called because the protein is associated with the pigments chlorophyll and peridinin, a type of carotenoid.

The structure shows that the peridinin and chlorophyll molecules are tightly packed within a vessel formed by the protein, an arrangement that allows for swift transfer of energy from the carotenoid, which captures the light, to the chlorophyll, which can then pass it on to the rest of the photosynthetic machinery. Says membrane biochemist Richard Cogdell of the University of Glasgow, Scotland, "It's a beautiful structure, aesthetically pleasing." Studies like this one, he notes, help explain why "there's a lot of interest in organisms that harvest light so well." Indeed, together with earlier structures of light-harvesting centers (LHCs) from other photosynthetic organisms, the results underscore the extent to which these complexes of molecules are tailored to each species' ecological niche.

It has, however, taken plant biochemists a full 20 years to begin to get an appreciation of the diversity of the LHCs, although chemical analyses provided some clues. Among other things, they showed that organisms in different environments may use different pigments, depending on the wavelength of light available. For example, *A. carterae*'s use of peridinin, which absorbs blue-green light, in the 470- to 550-nanometer range, is presumably an adaptation for collecting light in aquatic environments where light of that wavelength predominates. But a full understanding of how LHCs operate requires knowledge of how the pigments interact with each other and with the other components of the centers. And that information has been slow in coming, primarily because some structures are membrane-bound and difficult to isolate and prepare for crystallographic analysis using x-rays.



E. HOFMANN *ET AL.*

Light catcher. This *A. carterae* LHC contains three identical proteins (yellow-green), each with a cargo of chlorophyll (green), peridinin (red), and lipid (blue) molecules.

Indeed, the first two LHCs solved were not of the membrane-bound variety. In 1975, Brian Matthews of the University of Oregon, Eugene, and his colleagues obtained the structure of a soluble LHC, isolated from a species of green sulfur bacteria that lives at a depth of about 10 meters in lakes. And in 1985, Robert Huber and his colleagues at the Max Planck Institute in Munich, Germany, solved the x-ray structure of another soluble LHC, this one from the cyanobacterium *Mastigocladus laminosus*. These turned out to have distinctly different structures.

The sulfur bacterium's LHC, which absorbs blue light at about 460 nanometers