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A Tale of Two Senses

Meeting Review

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It has been about a decade since the molecular basis of the excitatory pathway in vertebrate photoreceptors was resolved. The lessons learned from that achievement have now been applied to other sensory modalities. The belief that each sensory modality relies upon unique, specialized molecules seems no longer tenable. Instead, common molecular components are adapted as needed and may function not only in sensory transduction but in axon outgrowth, desensitization, and trophic support of receptive cells. An appreciation of this commonality provided the incentive for the Boehringer Ingelheim Fonds International Conference on Signal Transduction in Vision and the Chemical Senses, held in Titisee, Germany, March 22–26, 1995. The success of this approach was demonstrated by the lively and critical discussions between individuals from differing sensory persuasions. Sessions were held on vertebrate and invertebrate photoreception, olfaction, and taste, as well as chemical signaling in unicellular organisms and sperm. Mutual interests extended far beyond the study of the family of receptor proteins with seven transmembrane domains, to include cyclic nucleotide–gated (CNG) channels, inositol-1,4,5-trisphosphate (IP_3) receptors, G protein receptor kinases, the arrestin gene family, and phosphodiesterases. However, investigations into each sensory modality are at different stages in their evolution. While much of the meeting debated mechanisms by which sensory receptors are activated, the vertebrate photoreceptor session centered upon turn-off and adaptation mechanisms. We will begin with a review of sessions dealing with how sensory receptors are excited.

Olfaction: One Transduction Pathway or Two?

A remarkable feature of olfaction is the diversity of chemical stimuli that can be discriminated. A question currently debated in research upon the chemical senses is the degree to which this is matched by a diversity of transduction pathways, in particular the roles of the cyclic nucleotide and phosphoinositide pathways.

The importance of cyclic nucleotides in sensory transduction was firmly established by the demonstration of cGMP-gated channels in membrane patches excised from the outer segments of vertebrate rod photoreceptors (Fesenko et al., 1985). The discovery of an odorant-sensitive adenylyl cyclase (Pace et al., 1985) and a CNG conductance in amphibian olfactory sensory cilia (Nakamura and Gold, 1987) extended this mechanism to olfaction and suggested a simple model for olfaction. Odorants activate adenylyl cyclase via a GTP-binding, protein-linked receptor; cAMP then activates CNG channels so as to produce a generator current. Much of the debate at the meeting concerned additions and alternatives to this model.

At about the same time as the discovery of CNG channels in rods, IP_3 emerged as a second messenger (Streb et al., 1983). It too was soon implicated in sensory transduction, but in invertebrate photoreceptors (Fein et al., 1984; Brown et al., 1984). Questions were soon raised as to whether IP_3 could also play a role in olfaction. Breer (Universitat Hohenheim) and Ronnett (Johns Hopkins Medical School) reported that odorants induce rapid and transient increases in both cAMP and IP_3 in isolated rat olfactory cilia. Immunoelectron microscopy localizes IP_3 receptors to the ciliary plasma membrane (Ronnett), where the components of the cAMP pathway have also been localized. What is not clear, however, is whether a single cell is capable of utilizing both pathways, or whether both can be activated by the same odorant receptor molecules. Breer reported that responses are mutually exclusive; odorants stimulate either one or the other signaling pathway. However, Ronnett reported that, in cultures of olfactory receptor neurons and in isolated rat olfactory cilia, all odorants tested elicited both a cAMP and an IP_3 response, but with different potencies. In addition, confocal light microscopy localized elements of the cAMP and IP_3 cascades uniformly across the ciliated epithelium, suggesting that they may have overlapping expression at the cellular level. The question of the use of multiple second messenger pathways in a single sensory cell is one to which we will return several times in this review.

If IP_3 is generated during olfactory stimulation, what does it do? Activation of an IP_3 receptor in the plasma membrane of olfactory neurons would have two obvious consequences: activation of calcium influx and elevation of intracellular calcium. As regards the former, it is unclear whether the current flowing through IP_3 receptors in the cilium depolarizes a receptor cell. However, even if the receptor has no direct effect on membrane potential, the elevation of intracellular calcium could modulate the responsiveness of olfactory cilia to odorants (see below). Involvement of the diacylglycerol pathway was also considered (Frings, Forschungszentrum Jülich Institiut für Biologische Informationsverbeitung). Thus, while the central role of cAMP appears clear, the role of IP_3 remains uncertain.

Odorant Receptor Specificity

In 1991, a family of putative odorant receptor genes was identified (Buck and Axel, 1991). One would hope that the distribution among cells of different odorant receptor types might eventually shed light on the activation of downstream transduction cascades. Thus far, groups of receptors have been found to demonstrate a zonal distribution across the neuroepithelium (Ressler et al., 1993). Most

receptor types are expressed in one of three broad zones, although a few subtypes are expressed in sensory neurons located in clusters at the tip of two of the turbinates (Breer). The true significance of this zonal expression for olfactory coding awaits the functional expression of these receptors. Without effective assays for binding, receptor specificity must be followed indirectly. Breer reported that cell lines expressing a single receptor are activated by a broad set of odorants. However, these results must be interpreted cautiously, as the receptors are being expressed in a heterologous system. Genetic approaches are being used to approach the problem of matching odorants with their cognate receptors. Reed (Johns Hopkins University School of Medicine) discussed strategies to permit the identification of loci involved in specific anosmia in strains of mice.

Multiple Functions of Calcium in Olfactory Transduction

Activation by odorants of either the cAMP or the phosphoinositide cascade could elevate intracellular calcium: the former through CNG channels and the latter through IP_3 receptors. Frings showed that the calcium permeability of the olfactory CNG channel is considerably higher than that of the rod channel, such that the inward current carried by the olfactory channel is almost a pure calcium current at 3 mM extracellular calcium. Thus, it appears that the CNG channels may function as triggers for other events. A comparison of calcium blockage/permeation in CNG channels with that of voltage-gated calcium channels reveals a number of common features, illustrating structural and functional similarities between these two channel families.

Once calcium enters the olfactory neuron, several modulatory events may occur. The most immediate effect of this increase in intracellular calcium is activation of an odorant-induced depolarizing chloride current (Kleene, 1993; Kurahashi and Yau, 1993), which contributes up to 85% of the total generator current (Lowe and Gold, 1993). An important consequence to this "ionic" cascade is that it introduces a threshold. Lowe (Monell Chemical Senses Center) presented evidence that this threshold attenuates transduction noise caused by spontaneous fluctuations in the basal cAMP concentration (Lowe and Gold, 1995). A lively discussion ensued as to whether these fluctuations are the source of the current bumps recently ascribed to the actions of single odorant molecules (Menini et al., 1995). Perhaps eventual estimation of the number of odorant receptors activated at a given odorant concentration will settle this debate.

Calcium influx via the CNG channels could also feed back to modulate the sensitivity of the cyclic nucleotide pathway by activating adenylyl cyclase and cAMP phosphodiesterase (Ronnett). A further, very powerful site of calcium feedback was described by Yau (Johns Hopkins University School of Medicine). The affinity of the olfactory CNG channel is decreased 20-fold by calcium–calmodulin at micromolar calcium concentrations. This effect appears to be important for olfactory adaptation. Previous experiments by Kurahashi and Shibuya (1990) showed that the generator current is maintained in the absence of extracellular calcium, but becomes transient in its presence. Further experiments by Kramer and Seigelbaum (1992) indicated that, in the presence of an endogenous soluble factor, calcium decreases the affinity of the olfactory CNG channel for cAMP. Yau's group has localized the calmodulin-binding site to the cytoplasmic N-terminus of the channel.

A Role for cGMP in Olfaction

A soluble and a particulate guanylyl cyclase may be involved in olfaction. Ronnett presented evidence to demonstrate an odorant-evoked rise in cGMP concentration, which was delayed and sustained when compared with cAMP and IP_3 signals. This suggests that cGMP may be involved in desensitization or long-term, activity-driven responses. This effect may be mediated by CO produced by the action of heme oxygenase. However, a contribution to olfactory cGMP levels could also be made by a novel membrane receptor guanylyl cyclase recently cloned from rat, termed GC-D (Fülle, University of Texas Southwestern Medical Center). This receptor is specifically expressed in a small subpopulation of olfactory receptor neurons, within a single zone of olfactory epithelium, resembling patterns typical of individual putative odorant receptors. The primary structure of GC-D is most homologous to two receptor guanylyl cyclases found in rat retina (GC-E and GC-F), thus apparently defining a new subfamily of sensory receptor guanylyl cyclases. When transiently expressed in COS cells, GC-D displays ligand-independent enzyme activity.

Invertebrate Olfaction: Establishment of General Principles

In addition to their intrinsic interest, presentations on invertebrate olfaction presented the meeting with a demonstration of fundamental principles that may be applicable to vertebrate modalities. Ache (University of Florida Whitney Laboratory) demonstrated that two second messenger pathways involving cAMP and IP_3 can, indeed, be functional in the same receptor cell. Odorants inhibit, as well as excite, olfactory receptor cells in some animals. In lobster olfactory receptor neurons, the inositol phosphate signaling cascade mediates excitation, while the cyclic nucleotide cascade mediates inhibition. The two signaling pathways target opposing conductances at the membrane level to determine the net potential of the cell. Activation of the phosphoinositide cascade by odorants targets neuronal plasma membrane IP_3 receptors. These receptors are functional nonspecific cation channels that depolarize the receptor cell when activated. Cyclic nucleotide–activated potassium channels mediate hyperpolarization and inhibition.

Carlson demonstrated that genetic screens of anosmic flies can yield novel information and stressed similarities in the genetic and molecular mechanisms of vision and olfaction. Carlson decribed several mutant Drosophila strains selected by a behavioral screen for anosmia. Two of these genes, *no receptor potential A* (*norpA*) and *retinal degeneration B* (*rdgB*), are also associated with the phos-

phoinositide cascade in invertebrate phototransduction. The mutants therefore provide evidence for the use of the IP_3 signal transduction pathway in Drosophila olfaction. *norpA* encodes a phospholipase C required for phototransduction (Bloomquist et al., 1988). *norpA* mutants that are null by genetic criteria are less sensitive to odorants. However, some physiological response remains, suggesting that some olfactory information may flow by a pathway independent of *norpA*. The *rdgB* gene, previously shown to encode a putative phosphatidyl inositol transfer protein in the retina (Vihtelic et al., 1993), is also required for normal olfactory as well as visual physiology. Carlson also presented data to demonstrate that, in Drosophila, nitric oxide synthase immunoreactivity is observed in the developing olfactory system as well as in some mature olfactory neurons, making its disposition different than that in the mammalian system. Additionally, Carlson has recently isolated an olfactory mutant identified by virtue of defective olfactory-driven behavior, which has reduced levels of nitric oxide synthase immunoreactivity in its olfactory organs, as well as reduced NADPH–diaphorase staining. The olfactory defect and the reduction in nitric oxide synthase levels both map to the X chromosome. It will be of interest to see whether this mutant has abnormalities in desensitization, as cGMP in the mature mammalian system may subserve such a role.

Taste: Defining the Pathways of Each Modality

It has been unclear as to whether more than one of the five known taste modalities—sweet, sour, bitter, salt, and unami—can exist within the same receptor cell. In addressing this problem, presentations on taste provided new examples of how signaling pathways for different classes of tastants can be compartmentalized. Lindemann (Universitat des Saarlandes) in collaboration with Bernhardt, Naim, and Zeheva (The Hebrew University of Jerusalem) examined the responses of cells that are sensitive to sweet tastants in the circumvallate taste buds of rats. The situation is complicated in that sweet-sensitive cells respond to sucrose and nonsugar sweeteners, such as saccharine, differently. Using calcium imaging technology, Lindemann presented evidence that a single sweet-sensitive cell can respond to sucrose with an increase in cAMP, closure of potassium channels, and calicum influx, but to nonsugar sweeteners with IP_3-induced calcium release. The increase in intracellular calcium common to both pathways may reflect the need for calcium in synaptic exocytosis or for signal termination. More intriguing, perhaps, is the need for the alternative pathways in the same cell. Sweet-sensitive cells did not respond to the bitter tastant denatonium chloride with an increase in intracellular calcium. Thus, in this example, sensory cells can respond to different classes of sweet molecules with different second messenger responses, but appear unable to respond to different taste modalities.

Margolskee (Roche Institute of Molecular Biology) proposed a model for bitter taste transduction and provided further evidence that transduction proteins used in vision have been adapted to other sensory modalities. In addition to the GTP-binding protein gustducin, rod transducin is expressed in rat and bovine taste cells. A taste receptor fraction isolated from bovine taste tissue activates both gustducin and transducin in the presence of denatonium, but not in the presence of saccharine, sucrose, or quinine. The target of transducin may be a phosphodiesterase. However, it is at this point that deviation from the visual pathway occurs: cyclic nucleotides act to suppress a calcium conductance in frog taste cells. This conductance may serve as an end target in the gustducin–transducin taste transduction cascade. A decrease in cyclic nucleotide levels following the arrival of a bitter tastant may therefore lead to an increase in the probability of the channel opening, allowing calcium entry and depolarization.

The ability to detect sodium is essential for all organisms. It is therefore perhaps not surprising that the sodium-specific transduction element in taste receptor cell membranes is a sodium channel. The sodium influx depolarizes the cell, leading to transmitter release at the basal surface. DeSimone (Virgina Commonwealth University) reported on this amiloride-sensitive sodium channel present in the apical membranes of taste cells. Like transporting epithelia, taste cells have apical and basal lateral membrane domains that are separated by tight junctions. Stimulation of these domains has been studied by recording responses to salts under voltage clamp. Results obtained from this protocol explain the pronounced effect of anions on the response to sodium and potassium.

To date, no receptor protein for taste has been isolated. In keeping with the emerging theme that molecules employed in sensory recognition may subserve other neuronal functions, Roper and Chaudhari (Colorado State University) presented an instance in which a molecule normally associated with synaptic transmission may subserve a sensory function. Experiments designed to identify receptors responsible for the taste of monosodium glutamate (MSG) revealed the presence of a metabotropic glutamate receptor, mGluR4, uniquely associated with taste buds. In situ hybridization demonstrated that mGluR4 is detectable in up to two-thirds of vallate and foliate taste buds, but not in surrounding, nonsensory epithelium. Expression of mGluR4 in taste buds is higher in preweaning rats compared with adults, perhaps corresponding to a known higher sensitivity to taste of MSG in juvenile rodents. Interestingly, free glutamate in the diet seems to regulate rat mGluR4 expression: rats weaned on a low glutamate diet retained a high level of mGluR4 expression in taste buds, whereas litter mates weaned on a high glutamate diet had a much lower expression of mGluR4 in their taste buds. Behavioral studies using a conditioned taste aversion test indicated that L-AP4, a specific ligand for mGluR4, mimics the taste of MSG in rat, whereas other glutamate receptor ligands showed very little overlap of their taste to that of MSG.

Invertebrate Photoreception: Searching for the Light-Activated Channels

The visual cascade in microvillar photoreceptors of invertebrates appears different from that of vertebrate rods or cones. Channels open rather than close in response to light, and the phototransducing membrane is often folded

into microvilli rather than discs. The field suffers from some of the same problems that beset the complete determination of pathways in olfaction and taste, namely difficulty in reconciling results obtained from different species and the possibility that more than one second messenger pathway may be involved within a single receptor. In the 1980's, work on Limulus photoreceptors pointed to the phosphoinositide pathway as the mediator of visual transduction. This hypothesis has been largely confirmed by work on what is now the best understood invertebrate visual system, that of Drosophila (Zuker, U. C. San Diego). Many mutants of Drosophila with impaired visual transduction have been isolated, and many of these are deficient in aspects of the phosphoinositide pathway.

However, neither genetic nor physiological approaches have yet confirmed the identity of a final messenger that opens channels in the plasma membrane, following activation of the phosphoinositide cascade by light. An obvious candidate is calcium released from stores by IP_3. In a continuation of work on Limulus ventral photoreceptors, evidence was presented that calcium release is required for the earliest phase of the response to light (Lisman, Brandeis University), and that confocal imaging of calcium signals can be used to detect release of calcium coincident with or, in some experiments, preceding the activation of ion channels in the plasma membrane (Payne, University of Maryland). Part of the paradox of visual transduction in Limulus is that the only known channel in the plasma membrane with similarities to the light-activated channel is activated not by calcium but rather by cGMP (Lisman). Given the work on vertebrates, Lisman proposed that modulation of cGMP metabolism by calcium may provide the missing link.

The evidence for activation of channels by calcium in Drosophila is even less substantial. Release of intracellular caged calcium does not activate the photocurrent directly, though it does strongly facilitate the response to light at early times, and adapt it later (Hardie, University of Cambridge). Two genes, *transient receptor potential* (*trp*) and its homolog, *trpl*, are promising candidates for a calcium channel protein or subunit. *trp* mutants are able to transduce, but with a drastically reduced calcium influx in response to light. Thus *trp* may either confer calcium permeability as a subunit of another, unknown light-sensitive channel (perhaps *trpl*), or it may form a calcium channel itself. If *trp* does form a channel, however, its sequence does not immediately suggest a binding site for an activator. As a possible solution to this conundrum, Hardie presented evidence that the light-activated current in Drosophila might be activated by depletion of calcium stores that underlie the phototransducing membrane. Exposure of Drosophila photoreceptors to calcium ionophore under certain conditions can trigger an inward current. To complete the hypothesis, light would have to deplete the stores via the phosphoinositide cascade.

The debate was broadened by the presentation of a new candidate for the light-sensitive channel in Drosophila, a homolog of the vertebrate cGMP-gated channel (Baumann, Forschungszentrum Jülich Institut für Biologische Informationsverbeitung) whose transcripts are found in PCR amplification performed on microdissected eyes and antennae. This channel has yet to be localized to the photoreceptive microvilli. A role for this channel in invertebrate visual transduction would require linkage of the phosphoinositide cascade to cGMP metabolism, as Lisman proposes for Limulus photoreceptors. Given this connection, the existence of this channel would provide an evolutionary link between Drosophila, Limulus, and vertebrate vision. Characterization of *trp*, *trpl*, and the CNG channel should provide new information in the near future. Mutants are eagerly awaited.

Reconciling these various models of visual transduction in invertebrates is a considerable challenge. One solution is to follow the lead suggested by the chemical senses and propose that all of the rival pathways are active, but at different light intensities and times. Nagy (Rheiniech-Westfalischen Technischen Hochschule, Aachen) presented evidence that the light response in Limulus ventral photoreceptors can be separated into different components with different pharmacological properties. He proposed that each component represents a different biochemical transduction cascade, with each cascade coupled to different channels types in the plasma membrane.

Vertebrate Vision: Adaptation and Turn-Off Reactions

Every activated stage in a transduction cascade must be rapidly turned off. Having essentially solved the problem of visual excitation, recent work on vertebrate rods and cones has concentrated on mechanisms of turn-off and adaptation. Perhaps the best understood turn-off reaction is the very first one, the termination of the catalytic activity of photoactivated rhodopsin. Phosphorylation of amino acids in the C-terminal region of rhodopsin, and the consequent binding of arrestin, effectively blocks further activation of the GTP-binding protein, transducin. Baylor (Stanford University) presented results obtained from mutant mice, in which a fraction of the rhodopsin molecules in each rod lacked the C-terminal region containing the phosphorylation sites. Consequently, a fraction of the single photon responses recorded from mutant photoreceptors was greatly prolonged. Aside from the elegant demonstration of the relevance of the phosphorylation reaction in vivo, new kinetic insights were obtained. Comparison with normal single photon responses suggested that phosphorylation of rhodopsin begins to turn off activation of the cascade before the peak of the electrophysiological response to a photon, and therefore limits the gain of transduction.

Of particular interest are turn-off reactions whose rates are accelerated during exposure to sustained illumination. This acceleration may result in the faster, less amplified responses typical of the light-adapted photoreceptor. Physiological recordings have indicated that the reduction in calcium ion concentration during sustained illumination is a necessary signal that initiates light adaptation. Thus, several talks concentrated on the modulation of the visual

transduction cascade by calcium ions. Light adaptation appears to act at several stages of the visual cascade. First, the availability of catalytic rhodopsin is reduced (Baylor), and the phosphorylation of rhodopsin is increased so as to inhibit the cascade at its inception (Kawamura, Keio University). The modulation of rhodopsin phosphorylation is now thought to be a function of the protein known as S-modulin, or recoverin (Kawamura). At high calcium concentrations, S-modulin inhibits the phosphorylation of rhodopsin, thus increasing the gain of the visual cascade in darkness, while reducing it in the light when calcium levels drop. The action of S-modulin does not appear to require its binding to the photoreceptive disc membranes and may involve regulation of soluble rhodopsin kinase activity. A second site of adaptation lies in the restoration of cGMP levels to prevent saturation of the photoreceptor during intense illumination. Two novel calcium-binding proteins have been isolated, guanylate cyclase–activating protein (GCAP; Palczewski, University of Washington) and a homolog, p24 (Hurley, University of Washington). These proteins activate guanylate cyclase at low calcium concentrations, and thus might be able to restore cGMP levels following intense illumination. In addition to adaptation of the level of cGMP, the sensitivity of the cGMP-gated channel itself can be modulated. The rod CNG channel turns out to be more complex than envisioned in the 1980's. The original 63 kDa α subunit (Kaupp et al., 1989) has been joined by a 240 kDa β subunit (Molday, University of British Columbia). This subunit binds calcium–calmodulin and confers a calcium sensitivity on the activation of the channel by cGMP. At low calcium levels, the channel is somewhat more sensitive to cGMP, allowing it to reopen during sustained illumination, even if cGMP levels remain low.

Given this multiplicity of calcium-sensitive modulation, one is tempted to ask whether all of the mechanisms discussed are physiologically relevant to light adaptation. There was debate concerning the calcium concentrations required to modulate activity of each mechanism in vitro, compared with in vivo calcium ion levels during sustained illumination. Estimates of both, however, may vary depending on methods and conditions. Perhaps, as with rhodopsin phosphorylation, specific mutations in mice will provide a more definitive answer.

New Horizons: Chemosensation in Sperm—Links to Olfaction

The arguments concerning compartmentalization of different transduction pathways in different cells are by necessity obviated in unicellular organisms and gametes. However, this fact does not simplify the task of understanding their transducing mechanisms.

Although it might be satisfying to apply the theories of chemotaxis to human sperm, the ability of human sperm to detect and respond to chemical cues has been controversial. Eisenbach (The Weizmann Institute of Science) reported that sperm chemotaxis indeed occurs in humans. Earlier studies carried out in vitro demonstrated human sperm attraction to follicular fluids. A correlation between sperm attraction and fertilization was implied, as one could only detect attractive activity in fluids from follicles in which the eggs had been fertilized. Investigations have now demonstrated the involvement of chemotaxis in this attraction. However, only 2%–12% of the sperm population are chemotactically responsive, and this responsiveness is transient. Interestingly, different sperm cells possess this responsiveness at different times. It had been known that mammalian sperm cannot fertilize the egg immediately after ejaculation, but that they require a period to become "capacitated." Eisenbach and his colleagues have found a correlation between capacitation and chemotaxis: the fraction of capacitated cells was 13-fold higher in a subpopulation enriched with chemotactic spermatozoa than in the rest of the population. In addition, there was a temporal correlation between the capacitated state and chemotactic responsiveness. These results indicate that the capacitated state of an individual spermatozoan is not static, as had previously been believed, but indeed rather temporary. Furthermore, the transient and synchronous nature of the capacitated state and the chemotactic responsiveness raise the possibility that sperm acquire chemotactic responsiveness only when they become capacitated, and lose it when this capacitated state is ended. In vivo, the role of sperm chemotaxis may be to select capacitated cells from the noncapacitated ones; by having waves of capacitation, this process can ensure the availability of capacitated spermatozoa over an extended period of time. The mechanisms of chemotaxis may now be addressed through application of the principals of chemosensory transduction at the molecular level to these cells.

The process of identifying molecules was addressed by Garbers (University of Texas Southwestern Medical Center) and Weyand (Forschungszentrum Jülich). Garbers reported on work defining gamete-specific molecules involved in mammalian fertilization. One sperm protein that appears to bind to egg extracellular matrix in a species-specific manner was isolated, and the cDNA was cloned. This protein is expressed by the haploid spermatid and not by other tissues. It contains a single putative transmembrane domain and five domains homologous to the D domains of von Willebrand factor. There is also a region that appears homologous to known mucins. This protein is localized to the apical part of the sperm head; more precise localization to the acrosome or to the plasma membrane is not yet available.

Weyand pursued similarities between gamete communication and sensory signaling. The CNG channel expressed in cone photoreceptors is also found in mammalian sperm. The channel in sperm was identified by patch-clamp recording, Western blotting, and immunohistochemistry. It is highly permeant to calcium and more sensitive to cGMP than cAMP. Peptides secreted from sea urchin eggs have been found to bind to membrane receptors of sperm, thereby leading to an increase in the intracellular concentration of cyclic nucleotides and to an influx of calcium. This rise in intracellular calcium has been postulated to be important in altering motility and the direction of sperm movement. Weyand speculated that these

CNG channels serve as the terminal component of a system that controls calcium entry, and therefore may be important to sperm chemotaxis. This finding supports the view that sperm utilize signaling components similar to those in photoreceptors and olfactory cells.

Unicellular Organisms: Novel Modes of Sensory Transduction

Using Paramecium as a model, Van Houten (University of Vermont) reported on a complex of three chemosensory transduction systems. Each has defined ligands that are small molecules put out by bacteria (their food stuff). All three attractant stimuli hyperpolarized these cells. Hyperpolarization increases the frequency of ciliary beating and reduces the frequency of action potentials that underlie the short reversal of ciliary beating, which produces a sharp turn during swimming. Thus, hyperpolarizing stimuli cause attraction by smooth, relatively fast swimming in a biased random walk manner. The first class of attractants is exemplified by acetate, folate, or extracellular cAMP. All of these stimuli cause hyperpolarization without increasing intracellular cAMP. The basis of these hyperpolarizations appears to be not channel activity, but rather an electrogenic pump activation. Molecular experiments have focused on the plasma membrane calcium pump that appears to demonstrate calmodulin dependence. The second pathway is activated by glutamate, which hyperpolarizes the cell and rapidly increases intracellular cAMP. This pathway seems to involve protein kinase A, as inhibitors of this kinase inhibit the chemoresponse to glutamate, but not acetate or ammonium chloride, the stimuli for the third pathway. In this last pathway, there is probably no cell surface receptor for the ligand; rather, ammonia appears to cross the membrane and raise intracellular pH, as has been measured directly using ion-sensitive fluorescent dyes.

A final warning against complacency was offered from a study of the unicellular alga, Chlamydomonas reinhardti, presented by Hegemann (Universität Regensburg). Unicellular algae such as Chlamydomonas reinhardti use a visual system for finding optimal light conditions for photosynthetic growth. The eye spot of this organism operates on the basis of an interference reflecting quarter wave stack. It is located in an equatorial position in the cell, thus providing directionality to the visual system. Chlamyrhodopsin, the functional photoreceptor pigment, contains an all-trans retinal chromophore, which is photo-isomerized from 13-trans to -cis. After flash stimulation, the photoreceptor channel of the eye spot is activated within 70 μs, leading to the assumption that the system lacks biochemical amplification. Thus, this system may be quite different from the other sensory signaling systems described, even in other unicellular organisms. In addition, chlamyopsin has been purified, and its gene has been sequenced. Based on this sequence, this opsin is more hydrophilic than any other rhodopsins and cannot necessarily be represented by a seven transmembrane domain receptor structure. Thus, the speculation is that this opsin, itself, may form a light-gated ion channel, or at least must form a complex with such a channel.

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References

Bloomquist, B. T., Shortridge, R. D., Schneuwly, S., Perdew, M., Montell, C., Steller, H., Rubin, G., and Pak, W. L. (1988). Isolation of a putative phospholipase C gene of Drosophila, *norpA*, and its role in phototransduction. Cell *54*, 723–733.

Brown, J. E., Rubin, L. J., Ghalayini, A. J., Tarver, A. L., Irvine, R. F., Berridge, M. J., and Anderson, R. E. (1984). Myo-inositol polyphosphate may be a messenger for visual excitation in *Limulus* photoreceptors. Nature *311*, 160–162.

Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell *65*, 175–187.

Fein, A., Payne, R., Corson, D. W., Berridge, M. J., and Irvine, R. F. (1984). Photoreceptor excitation and adaptation by inositol 1,4,5-trisphosphate. Nature *311*, 157–160.

Fesenko, E. E., Kolesnikov, S. S., and Lyubarsky, A. L. (1985). Induction by cGMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature *313*, 310–313.

Kaupp, U. B., Niidome, T., Tanabe, T., Terada, S., Bonigk, W., Stühmer, W., Cook, N. J., Kangawa, K., Matsuo, H., Hirose, T., Miyata, T., and Numa, S. (1989). Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. Nature *342*, 762–766.

Kleene, S. J. (1993). Origin of the chloride current in olfactory transduction. Neuron *11*, 123–132.

Kramer, R. H., and Siegelbaum, S. A. (1992). Intracellular Ca^{2+} regulates the sensitivity of cyclic nucleotide–gated channels in olfactory receptor neurons. Neuron *9*, 897–906.

Kurahashi, T., and Shibuya, T. (1990). Ca^{2+}-dependent adaptive properties in the solitary olfactory receptor cell of the newt. Brain Res. *515*, 261–268.

Kurahashi, T., and Yau, K.-W. (1993). Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. Nature *363*, 71–74.

Lowe, G., and Gold, G. H. (1993). Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. Nature *366*, 283–286.

Menini, A., Picco, E., and Firestein, S. (1995). Quantal-like channel fluctuations induced by odorants in olfactory receptor cells. Nature *373*, 435–437.

Nakamura, T., and Gold, G. H. (1987). A cyclic-nucleotide gated conductance in olfactory receptor cilia. Nature *325*, 442–444.

Pace, U., Hanski, E., Salomon, Y., and Lancet, D. (1985). Odorant-sensitive adenylate cyclase may mediate olfactory reception. Nature *316*, 255–258.

Ressler, K. J., Sullivan, S. L., and Buck, L. B. (1993). A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell *73*, 597–609.

Streb, H., Irvine, R. F., Berridge, M. J., and Schulz, I. (1983). Release of Ca^{2+} from nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature *306*, 67–69.

Vihtelic, T. S., Goebl, M., Mulligan, S., O'Tousa, J. E., and Hyde, D. R. (1993). Localization of *Drosophila* retinal degeneration B: a membrane-associated PI transfer protein. J. Cell Biol. *122*, 1013–1022.