disrupt caveolae effectively block bacterial uptake. The mast cell CD48 receptor also appears to be intrinsically associated with caveolin- and cholesterol-rich domains during the internalization process. Interactions between cholera toxin B and its receptor G_{M1}, an integral component of caveolae (*19*), specifically interferes with bacterial uptake, apparently by usurping caveolae-like domains in the host plasma membrane. These findings suggest that cholera toxin B and *E. coli* that express FimH are internalized in similar ways, despite the fact that they adhere to different host cell GPI-anchored receptors. The Shin report indicates that caveolae-like regions in the mast cell plasma membrane are highly dynamic elements and that GPI-anchored receptors can transmit signals and activate host cell endocytic pathways through association with caveolae-like membrane domains.

The potential of FimH-expressing *E. coli* to co-opt caveolae-mediated endocytic pathways through interactions with CD48 implicates caveolae-related domains in the pathogenesis of certain bacteria. CD48 and other GPI-anchored proteins belong to an expanding class of receptors for various viruses, bacteria, and bacterial toxins. The entry of simian virus 40 into host cells and the uptake of the bacterium *Campylobacter jejuni* by cultured intestinal epithelial cells both depend on caveolae-like membrane domains (*15*, *16*). Caveolae do not appear to fuse with endocytic vesicles (*20*), and so internalization of bacteria through caveolae-like domains could conceivably facilitate their intracellular survival. Indeed, uptake of FimH-expressing *E. coli* by macrophages (a process that is also dependent on CD48 and possibly caveolae) seems to enhance bacterial survival within these immune effector cells (*17*).

The consequences of FimH-dependent interactions of bacteria with mast cells in vivo are unclear. Mast cells are long-lived, heterogeneous immune cells that are strategically situated at sites of microbial entry and are thought to be important for innate host defense. Upon recognizing invading microbes, mast cells become activated and release bactericidal compounds and proinflammatory molecules. The interactions of FimH with CD48 and caveolae-like membrane domains possibly could direct bacteria to nonbactericidal compartments within mast cells, providing them with an obvious survival advantage. Paradoxically, it has been reported that FimH-expressing *E. coli* can specifically enhance mast cell bactericidal activity and the release of inflammatory mediators (*4*, *21*). It will be interesting to learn whether FimH-expressing pathogenic bacteria, once inside the host cell, can modulate its activation and so potentially dampen the antimicrobial response. The importance of caveolin and caveolae-like membrane domains in the uptake of FimH-expressing *E. coli* by mast cells and other cell types awaits further clarification. It appears that the mysterious recesses of caveolae will keep researchers spelunking further into the depths of these fascinating cellular domains for years to come.

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PERSPECTIVES: STRUCTURE

Rhodopsin Sees the Light

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Seven-helix transmembrane receptors are signal detectors that activate heterotrimeric GTP-binding proteins (G proteins) in response to extracellular stimuli. Genes encoding this huge family of G protein–coupled receptors (GPCRs) occupy a hefty 5% of the worm genome and perhaps 3% of our own. GPCRs have cornered much of the signal-transducing market because their shared three-dimensional (3D) architecture, based on a transmembrane bundle of seven α helices, can be adapted to detect diverse extracellular stimuli—hormones, neurotransmitters, odorants, even photons. These receptors transmit signals specific for each extracellular stimulus across the membrane lipid bilayer by selectively activating different G proteins. On page 739 of this issue, Palczewski *et al.* (*1*) report the first 3D structure of a GPCR at 2.8 Å resolution. The x-ray crystal structure of rhodopsin—the light-detecting GPCR found in rod cells of the retina that signals through the G-protein trimer, G_t—is sure to evoke widespread excitement among investigators who want to know how GPCRs transduce signals.

In a number of GPCRs the activating extracellular stimulus (ligand) has been found to occupy a binding pocket within the bundle of seven α helices, in the plane of the lipid bilayer. Somehow, ligand occupancy of the pocket induces rearrangements of the α helices, which in turn alter the shape of the receptor's cytoplasmic surface, thus activating the appropriate G proteins. The rhodopsin structure brings these events into sharp focus. Until now, bouncing notions back and forth about how GPCRs work was like playing tennis without a net. With the new net provided by Palczewski *et al.* the game will be harder, but also more fun.

Rhodopsin, unlike most GPCRs, binds its ligand (retinal) covalently (to lysine-296 in helix VII), both in the inactive (dark) state (represented by the new 3D structure) and also after photoactivation. Spectroscopic observations have shown that a photon causes the inactive ligand, 11-*cis*-retinal, to change into the all-*trans* isomeric form, which activates the rhodopsin receptor. Now we can see the chromophore's inactive form cradled in a pocket (see the figure) formed by transmembrane helices and by an elaborate, multilayered plug that comprises most of the receptor's extracellular domain. All elements of this domain contribute to the plug, which in fact contacts the chromophore. It is already established that the α-helix bundle forms the walls of the retinal-binding pocket, but an extracellular plug blocking exit from the pocket is a real surprise. Similar plugs will probably not be found in most other GPCRs because their ligands (which are reversibly bound) enter and leave the binding pocket in milliseconds.

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Why did evolution go to so much trouble to prevent escape of a ligand that is already covalently attached to rhodopsin? We speculate that the plug enhances the efficiency of rhodopsin's switch mechanism by preventing the photoactivated all-*trans* chromophore from projecting out of the pocket into the extracellular environment. In this way the plug may help to ensure that the strain energy created by the 11-*cis* to all-*trans* transition is efficiently converted into light-dependent rearrangement of surrounding helices. In other words, the plug allows the photon to flip the GPCR switch more easily and perhaps faster.

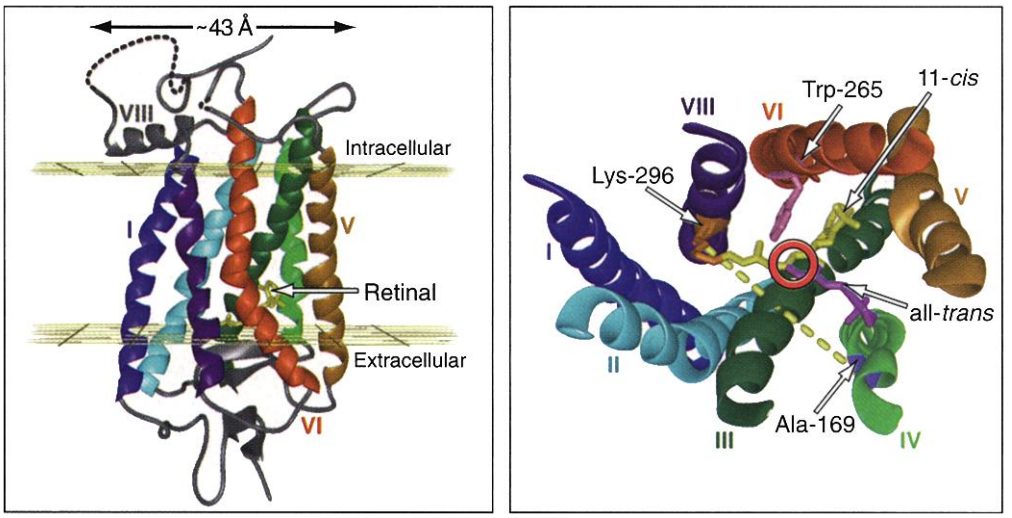
A recent cross-linking experiment (*2*) hints that all-*trans*-retinal does in fact remain within the α-helix bundle and suggests how this photoactivated chromophore may affect the conformation of the helices. In the dark, the cross-linking group located on retinal's ionone ring is attached to tryptophan-265 in helix VI—just as the crystal structure predicts (see the figure, right panel; 11-*cis*-retinal is yellow, tryptophan-265 is pink). In the light, however, the ionone ring is cross-linked to alanine-169 in helix IV—a linkage that appears unlikely in dark rhodopsin (see the figure; all-*trans*-retinal is magenta). Twisted (by computer) into the all-*trans* form, retinal does project its ionone ring toward helix IV of the dark structure, but alanine-169 in dark rhodopsin is located on the wrong side of the helix. Adjusting torsion angles in lysine-296 could point the chromophore more directly toward the alanine (along the dashed yellow line in the figure), but the chromophore in this position would clash with helix III.

Thus, if the cross-linking result is correct, activation of rhodopsin involves substantial movements of helices III and/or IV, and probably other helices as well. Indeed, site-directed spin labels, cross-linking approaches, and mutations in rhodopsin and other GPCRs have already suggested that receptor activation causes the cytoplasmic ends of helices III and VI to separate from one another [for example, (*3*, *4*)]. Similar approaches, guided by the new rhodopsin structure, will provide detailed understanding of the GPCR switch.

The 3D structures of GPCR cytoplasmic domains, which discriminate among and activate specific G proteins, were previously shrouded in mystery. Now, rhodopsin's entire cytoplasmic domain—three intracellular loops and a carboxyl-terminal sequence—presents intriguing surprises. The second intracellular loop and a short α helix (helix VIII) of the carboxyl terminus project laterally from either side of the receptor, parallel to the membrane's cytoplasmic face (see the figure, left panel). These projections create a platform (longest dimension, 43 Å) that may be broad enough to allow rhodopsin to interact simultaneously with carboxyl termini of the G_t trimer's α and γ subunits (which interact with rhodopsin and lie 40 Å distant from one another in the G_t crystal structure) (*5*). Peptides corresponding to intracellular loops and helix VIII are reported to prevent rhodopsin from activating G_t (*6*). It is not clear, however, which G-protein subunit interacts with helix VIII; different laboratories report that the cognate peptide interacts with the carboxyl terminus of either α_t (*6*) or γ_t (*7*).

Paradoxically, the least surprising part of the new rhodopsin structure—the seven-helix bundle—will generate considerable excitement among GPCR aficionados. The bundle is not altogether surprising because theoretical models had already predicted its structure. The very low resolution of previous cryoelectron micrograph structures (*8*) crudely indicated tilts and orientations of rhodopsin's transmembrane helices. To assign the densities to specific helices and to locate specific residues in each helix, modelers depended entirely on inference from the effects of many GPCR mutations and from comparing primary structures of hundreds of other GPCRs. Three models of the helix bundle (*9*–*11*) superimpose reasonably well on the actual crystal structure (root mean square deviation for α carbons, 3.1 to 3.2 Å). This accuracy supports a reciprocal inference: Transmembrane helices in the crystal structure of rhodopsin reliably predict the 3D architecture of the huge GPCR family.

The new high-definition snapshot of rhodopsin changes our images of the GPCR family as well. It constrains possible scenarios of how helices move once the receptor is activated and how G-protein trimers are activated, opening many new avenues for experiment. Elucidating the molecular mechanisms of receptor activation that are shared by the GPCR family should have far-reaching implications. New insights gained will help us to understand how GPCRs transduce the signals that regulate embryonic development and control the heart, blood vessels, endocrine responses, synaptic traffic in the brain and, indeed, the functions of virtually every eukaryotic cell.



Two views of rhodopsin. (Left) The seven α helices of the GPCR rhodopsin weave back and forth through the membrane lipid bilayer (yellow lines) from the extracellular environment (bottom) to the cytoplasm (top). **(Right)** The chromophore, retinal, is nestled among the transmembrane helices (loops not shown; enlargement viewed from the cytoplasm). Retinal is shown in its 11-*cis* dark form (yellow) and its all-*trans* light form (magenta, where the two differ). Roman numerals indicate numbered helices; the red circle indicates the position about which isomerization of retinal (from *cis* to *trans*) occurs.

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