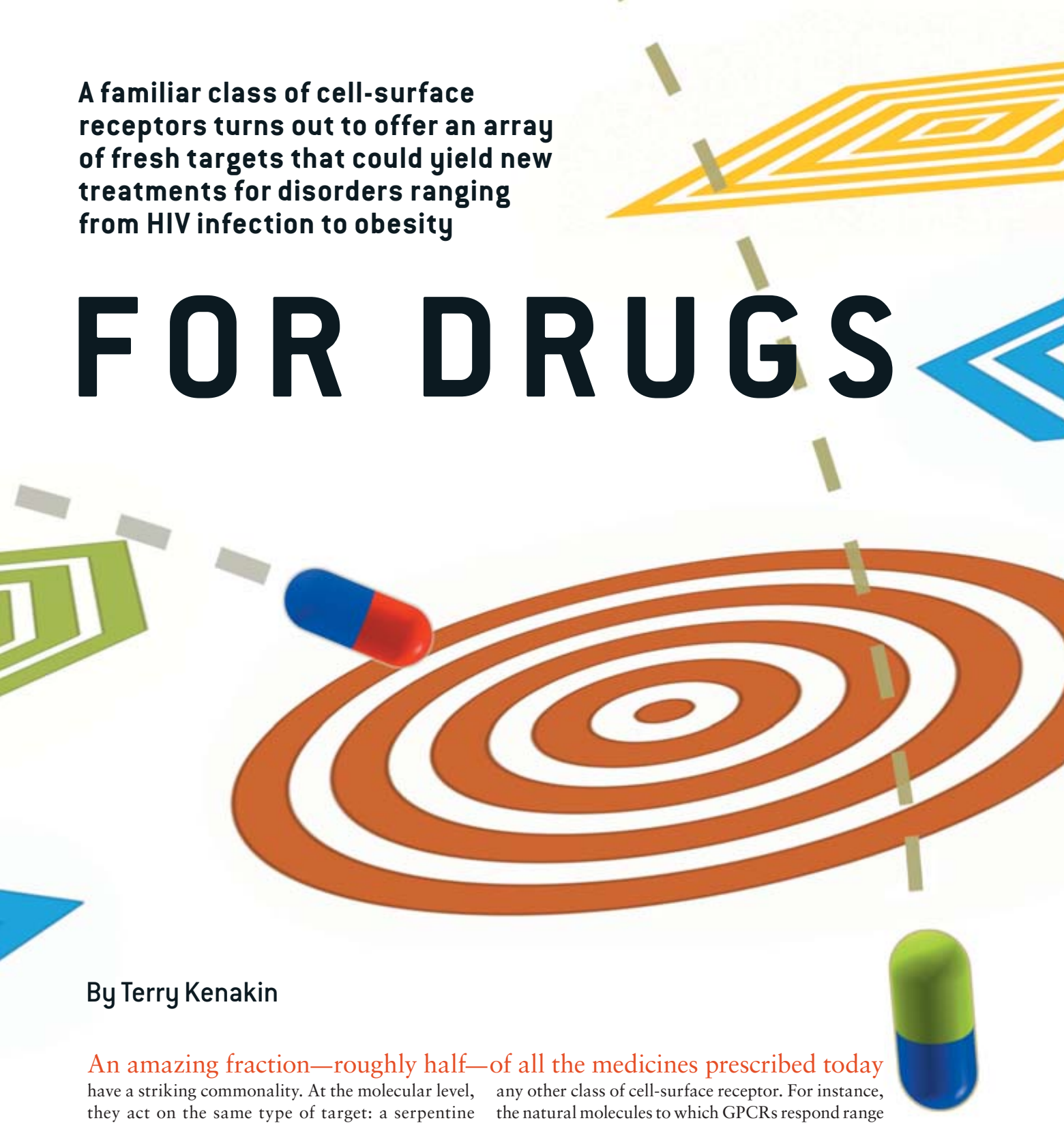
NEW BULL'S-EYES

A familiar class of cell-surface receptors turns out to offer an array of fresh targets that could yield new treatments for disorders ranging from HIV infection to obesity

FOR DRUGS



By Terry Kenakin

An amazing fraction—roughly half—of all the medicines prescribed today have a striking commonality. At the molecular level, they act on the same type of target: a serpentine protein that weaves seven times through the membrane that envelops the cell. External parts of each serpent serve as an antenna for molecular signals approaching the cell, and internal parts trigger the cell's responses to such cues, beginning with the activation of a signal processor called a G-protein. The serpents themselves are thus known as G-protein coupled receptors, or GPCRs.

As a group, GPCRs show far more versatility than any other class of cell-surface receptor. For instance, the natural molecules to which GPCRs respond range in size from neurotransmitters that are only a few times as massive as a single carbon atom all the way up to proteins 75 times larger than that. Moreover, GPCRs participate in just about every bodily function that sustains life, from heartbeat and digestion to breathing and brain activity. The drugs that target these receptors are equally diverse. The list includes blood pressure reducers (such as propranolol), stomach acid suppressors (such as ranitidine), bronchodi-

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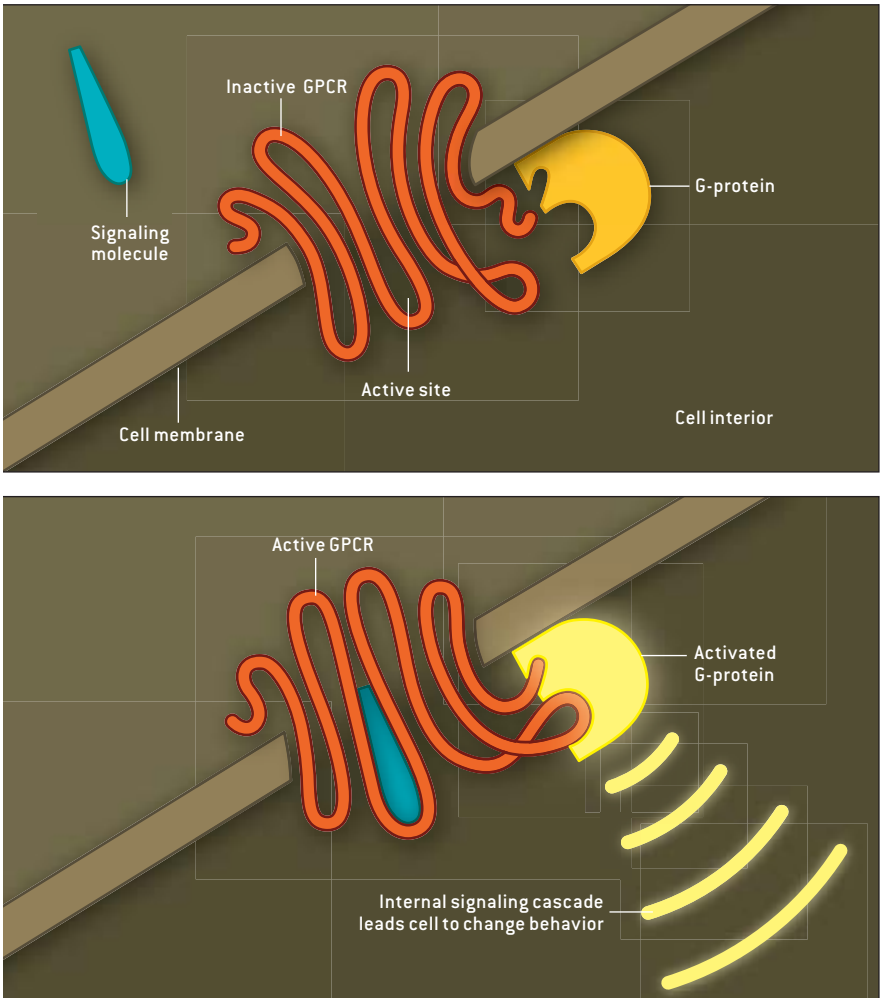

G-PROTEIN COUPLED RECEPTOR (GPCR), which snakes through the cell membrane seven times, typically issues no messages into a cell (*top*) until a signaling molecule, such as a hormone or a neurotransmitter, binds to a region called the active site. This binding (*bottom*) causes the receptor to activate a molecule called a G-protein, which triggers a series of intracellular interactions culminating in a change in the cell's behavior. New insights into the functioning of GPCRs suggest novel avenues for treating disease.

lators (such as albuterol) and antidepressants (such as paroxetine). The disorders these medicines treat include hypertension, congestive heart failure, ulcer, asthma, anxiety, allergy, cancer, migraine and Parkinson's disease.

Incredibly, today's GPCR-targeting drugs all work in one of two ways—they either attach to the "antenna" region of the receptor (also known as the active site) and mimic the effect of the natural neurotransmitter, hormone or other molecule that normally signals through the GPCR, or they interfere with a natural signaler's ability to act on the antenna. Over the past 15 years, a technological revolution has furnished investigators with new eyes with which to see GPCRs at work. Consequently, other ways of manipulating GPCR activity have emerged and are beginning to be mined for drug discovery. In other words, despite the wealth of medicines already known to act on these fascinating receptors, many more may lie ahead. The search for such pharmaceuticals is still in early stages, but a few agents, including some for HIV infection (the cause of AIDS), are now advancing through human trials.

Overview/*New Drug Targets*

- Proteins called G-protein coupled receptors (GPCRs), which sit on the cell surface, convey signals from hormones and the like into cells by activating G-proteins—signal processors residing just under the cell membrane.
- About half of all pharmaceuticals on the market act on GPCRs, binding to the sites normally targeted by the body's own extracellular signalers.
- In the past 10 years, researchers have learned that GPCR activity can also be modulated by compounds that bind to other sites on GPCRs. This discovery opens new possibilities for treating cancer and other major disorders.

Shape Matters

UNTIL ABOUT 10 YEARS AGO, pharmaceutical researchers thought that to influence the activity of GPCRs they would have to aim drugs at a receptor's active site. During the body's normal operation, a neurotransmitter or other information-bearing molecule (or "ligand") at the cell's outer surface essentially plays the "key" to the active site's "lock." So a substance that plugged the lock could prevent unwanted signaling through the receptor by any key and serve as an inhibitor. Conversely, something that mimicked the natural ligand could essentially open the lock and therefore take the place of the natural key if it were missing.

Scientists thought as well that the best way to evoke a selected physiological response was to choose a compound that interacted with a specific form of a receptor but ignored other variants. The neurotransmitter norepinephrine, for instance, activates two types of GPCR, called alpha and beta adrenoceptors, of which the first has four subtypes and the second has three. These various receptors, in turn, govern different life-sustaining processes. In the heart, $beta_1$ adrenoceptors quicken the heart rate and increase the force of each beat; in the lungs, $beta_2$ adrenoceptors widen the air passageways. Hence, to open constricted airways without unwanted

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effects on the heart, pharmaceutical makers might seek an agent that mimicked norephinephrine's ability to stimulate beta$_2$ adrenoceptors but without binding to beta$_1$ adrenoceptors.

Many medicines do, in fact, function as inhibitors or agonists (mimics) by interacting with the active site of a specific GPCR. But an emerging drug development strategy has to do with the "allosteric" nature of GPCRs: the shape of one part of the receptor can affect the conformation, and thus the activity, of a distant part.

GPCRs constantly adopt somewhat different shapes, essentially sampling a library of conformations. When a natural signaling molecule binds to the active site, it stabilizes the arrangement that activates G-proteins. But it turns out that certain molecules, known as allosteric modulators, can bind elsewhere to influence form and activity. Some stabilize GPCR conformations that promote signaling, whereas others maintain shapes that impede it (say, by burying the active site so that it becomes inaccessible to its natural ligand).

The implications are profound. The entire receptor can theoretically offer binding sites, at any one of which a diminutive molecule might stabilize a shape that yields some biological effect. This property greatly enlarges the vista for therapeutic modification of GPCR function.

AIDS researchers are among those actively pursuing the potential of allosteric modulators, trying to find ones able to block HIV from infecting cells. Biologists have long known that the virus attacks cells called helper T lymphocytes by adhering to a cell-surface protein named CD4. But in the mid-1990s they learned that this protein does not act alone.

To enter cells, the virus also has to bind to an additional anchor: a GPCR known as CCR5 (or, in late-stage infection, a GPCR called CXCR4). Normally CCR5 responds to any of three chemokines, natural signals that can attract immune system cells to a site of infection. Unfortunately, it also offers a hook for the virus's coat protein (gp120). Indeed, CCR5 now appears to be a central player in HIV infection; people whose genetic makeup causes them to lack a functional form tend to be extraordinarily resistant to HIV.

Several allosteric modulators that hold CCR5 in a shape inimical to binding by HIV's gp120 have already reached human trials. Blocking the gp120-CCR5 interaction by delivering these tiny drugs is an achievement comparable to, in a geophysical analogy, an island the size of Fiji preventing two Australias from coming together. In more allegorical terms, if such drugs work they will be the David that smites Goliath.

Beyond Volume Control

THE EFFECTS produced by GPCRs depend not only on the extracellular molecules that bind to them but also on how many copies of the receptors are accessible on the cell surface. As might be expected, when extracellular signalers bind to many copies of a receptor, the cell receives a "louder" message and undergoes a more pronounced behavioral change than when few copies of the receptor are bound. But the number of receptors can do more than control "volume." It can actually influence which of several G-protein species become stimulated and can thereby lead to activation of distinct pathways (cascades of molecular interactions) inside a cell.

G-proteins come in four major forms, with subtypes in each class. Each has a different proclivity for working with any given GPCR, and for its part a GPCR may not be equally active toward all G-proteins. A scant supply of a given receptor might therefore result in activation of only the most sensitive G-protein, whereas a greater abundance might lead to responses by multiple G-proteins, eliciting a different cellular behavior.

Accordingly, a GPCR can no longer be seen as simply a toggle switch turned on by a hormone or neurotransmitter and turned off when the natural signal diffuses away from its binding site. It is a much more sophisticated information-processing unit.

Theoretically, the variety of response patterns a given GPCR can generate will

MARKETED DRUGS ACTING ON GPCRs

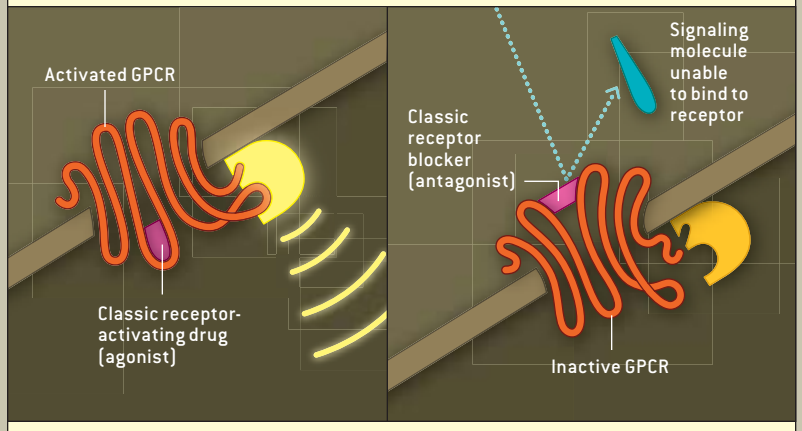
The products listed below are just a sampling of marketed compounds targeting GPCRs; they act on various receptors.

BRAND NAME (GENERIC NAME) AND MAKER	EFFECT
Allegra (fexofenadine) *Aventis*	Blocks histamine action, to control allergic responses
Duragesic (fentanyl) *Janssen*	Relieves pain
Flomax (tamsulosin) *Boehringer Ingelheim*	Eases symptoms of enlarged prostate
Imitrex (sumatriptan) *GlaxoSmithKline*	Eases migraines
Lopressor (metoprolol) *Novartis*	Lowers blood pressure
Oxycontin (oxycodone) *Purdue*	Relieves pain
Pepcid (famotidine) *Merck*	Counteracts stomach acid
Phenergan (promethazine) *Wyeth*	Blocks histamine
Serevent (salmeterol) *GlaxoSmithKline*	Opens airways
Singulair (montelukast) *Merck*	Controls airway inflammation
Sudafed (pseudoephedrine) *Pfizer*	Eases nasal congestion
Zantac (ranitidine) *GlaxoSmithKline*	Counteracts stomach acid
Zyrtec (cetirizine) *Pfizer*	Blocks histamine
Zyprexa (olanzapine) *Eli Lilly*	Eases symptoms of various psychoses

MANY AVENUES OF ATTACK

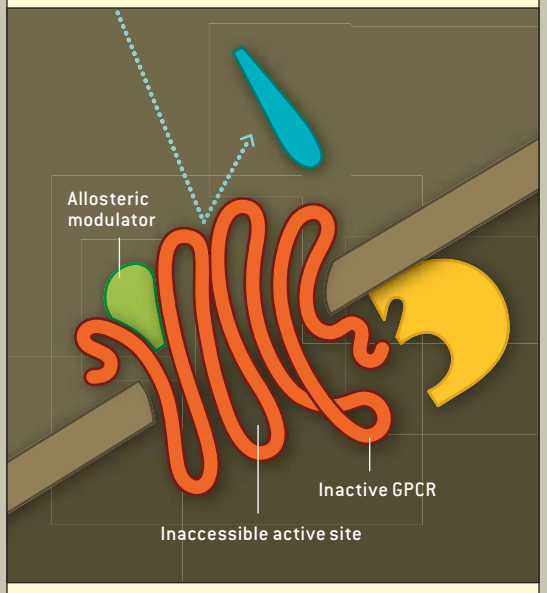
Most drugs on the market target the active site of some cell-surface receptor, and many aim for the active site of a specific GPCR (*below*). Yet molecules acting at regions outside the active site can also influence GPCR activity (*right*). Recent studies encourage hope that small molecules targeted to those additional sites could be administered to activate or quiet GPCRs involved in various diseases.

STANDARD DRUGS



Pharmaceuticals that act on GPCRs today usually fit into the active site and either mimic the action of a natural signaling molecule (*left*) or prevent the native signaler from docking with the receptor and thus from acting on a cell (*right*).

ALLOSTERIC MODULATORS



These agents stabilize the conformation of a GPCR in a way that enhances (*not shown*) or decreases (*above*) the receptor's activity, such as by causing the active site to become inaccessible to a signaling molecule.

depend on both the range of ligands it can detect and the mix of G-protein species it can activate. If, for example, a receptor can detect any of three different signals and can activate any one, two, three or all four of the major G-proteins (as is known to be the case for the GPCR responsive to thyrotropin, the pituitary hormone that stimulates the thyroid gland), the receptor gains the theoretical capacity for dozens of forms of behavior, each seen at one time or another. If it were only a toggle switch, it could have only two.

Research also suggests that drugs can take advantage of this complexity in receptor function. Distinct substances might cause a receptor to hold different biologically active shapes, each of which might interact with a distinct G-protein or G-protein combination, triggering the activity of divergent intracellular paths. Agents that can cause cells to increase or decrease the quantity of receptors at the surface, rather than altering GPCR activity per se, should be valuable as well.

This last strategy could be pursued for combating HIV. One problem that might arise from relying on allosteric modulators to prevent the viral coat protein from finding its docking site on CCR5 is that the virus mutates rapidly. This mutability could lead to the creation of a coat protein that would bind quite well to an allosterically altered CCR5. A plausible way to avert this threat would be to banish the receptor from the cell surface, thereby denying the virus its point of attack.

Like all other GPCRs, CCR5 is synthesized endlessly by the cell, stationed at the surface and then drawn back inside for degradation or recycling. And certain chemokines are known to promote CCR5 internalization. This observation raises the possibility of finding pharmacological agents that would not only accelerate the removal of CCR5 from the cell surface but would also serve as therapies to which the virus could not adapt. After all, no change that HIV could undergo would enable it to latch onto CCR5 if that receptor were removed from the cell surface.

Stopping Renegade Signaling

BEYOND BEING controllable by allosteric modulators, GPCRs may exhibit another biologically important behavior, known as constitutive activity—that is, sometimes they activate G-proteins even without being "told" to do so by a bound ligand. As is true in other forms of GPCR functioning, this one arises from a particular shape in the receptor's repertoire. The conformation, however, is one that the receptor rarely takes. Under normal circumstances, the number of molecules that adopt it will therefore be quite small, and so they will have little effect on the cell's overall behavior and will be hard to detect. But if the constitutively active

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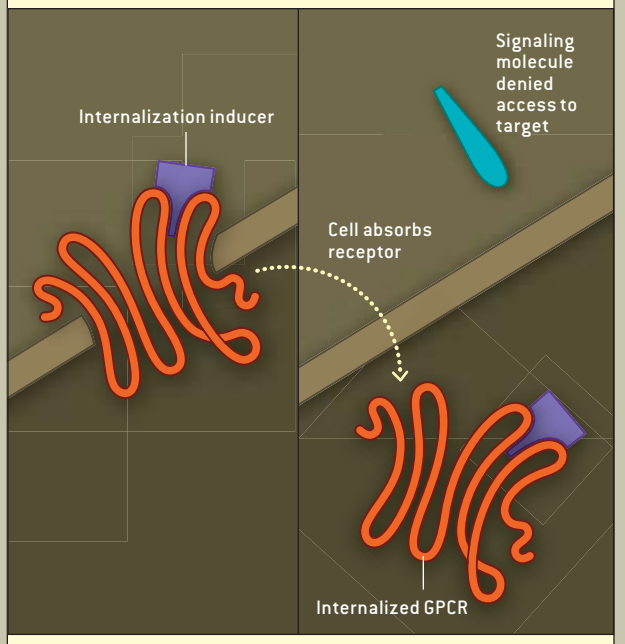
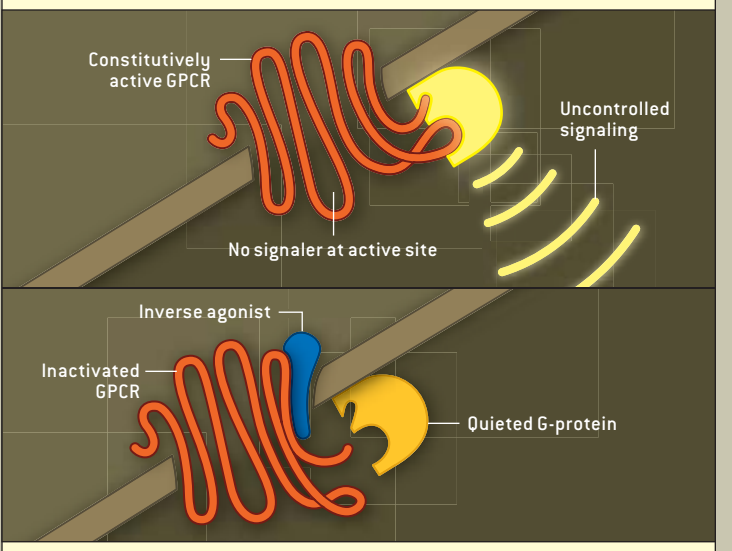
INDUCERS OF INTERNALIZATION



Internalization inducers cause receptors to leave the cell surface. They thereby block signal delivery into the cell.

INVERSE AGONISTS



Inverse agonists take aim at "constitutively" active GPCRs. Such receptors improperly behave as if they are bound by a stimulant even when they are not (*top*). Attachment of the inverse agonist quiets the signaling (*bottom*). Cancer cells often have high numbers of constitutively active receptors that theoretically could fuel uncontrolled cell division; inverse agonists, therefore, could potentially become a new form of cancer treatment.

receptors become sufficiently abundant, their combined signaling can exert a powerful influence.

The consequences become especially dramatic in illnesses such as viral infection or cancer, which may advance by inducing one or another receptor to behave in ways that promote the disease. In a form of pancreatic cancer, for example, the receptor for a hormone called vasoactive intestinal peptide (VIP) might be such a bad actor.

In a normal pancreatic cell that displays this GPCR, activation of the receptor by VIP supports cell division. But in people afflicted by this malignancy, the receptor becomes overabundant and the versions that act independently, without need for VIP stimulation, become correspondingly numerous—together they acquire the capacity for driving unconstrained proliferation of tumor cells. Oncologists have long been familiar with destructive constitutive activity in certain non-GPCR receptors, notably one called ras. In those cases, though, mutations in the receptor, rather than an aberrant plentitude of receptors, account for the behavior.

Standard pharmaceuticals cannot quell the cellular misbehavior triggered by constitutively active receptors. A conventional receptor stimulant, or agonist, would only cause more receptors to hold an active shape, to the patient's detriment. A conventional receptor blocker, or antagonist, might prevent natural signals from activating receptors, but such agents will have no effect on receptors that need no outside prompting in order to act. Thus, a new kind of drug is required, one that forces constitutively active GPCRs to maintain an inactive shape.

Such agents, called inverse agonists, might one day constitute an important new form of cancer therapy. They are also being eyed for treating obesity. In this realm, the envisioned targets include the receptor for ghrelin, a recently discovered hormone produced chiefly by the stomach, and the H_3 subtype of histamine receptor; both receptors appear to participate in the brain's regulation of appetite.

Exploiting Phantom Genes

AT LEAST ONE OTHER FORM of GPCR behavior remains to be mined for drug discovery. Cells sometimes mix and match proteins, forming complexes that function as receptors having sensitivities not seen in the individual components. In the most extreme form of this activity, the cell gains a responsiveness to a signal

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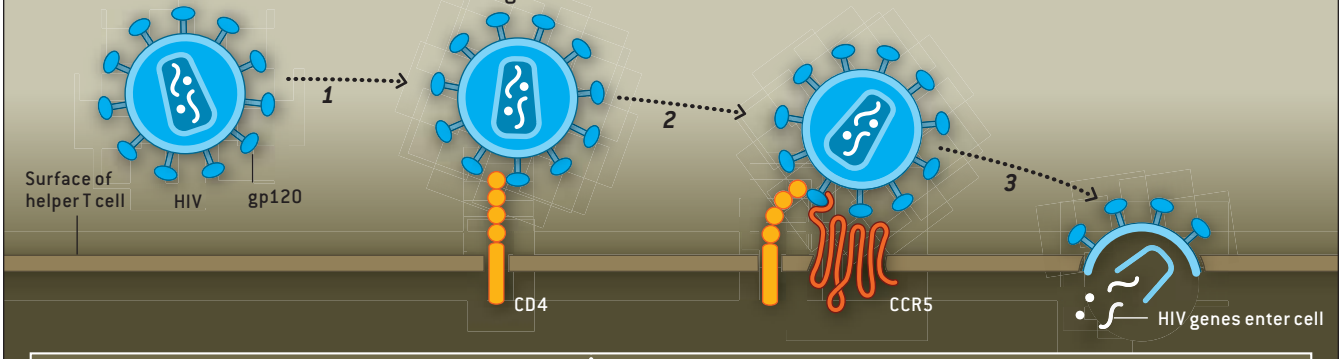
it would otherwise ignore. Individual proteins have their blueprints in specific genes, but these combination receptors have no corresponding single blueprint (from which their behaviors might be predicted), so they might be thought of as products of "phantom" genes.

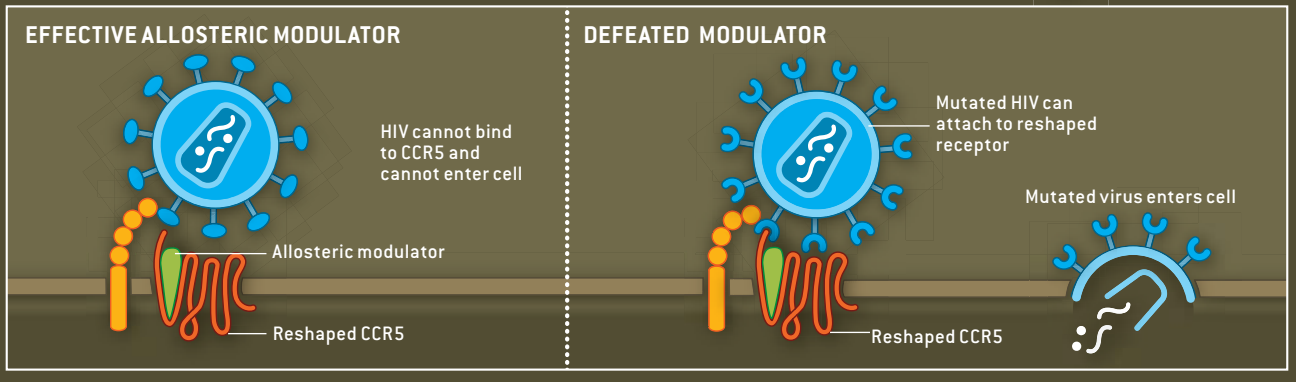
In some cases, the novel receptor is a complex consisting of two or more GPCRs. In other cases, it consists of a GPCR and a co-protein—one that is not itself a receptor but gives the receptor an altered set of properties. The receptor for a hormone called amylin seems to be of this type. Released by the same pancreatic cells that secrete insulin, amylin modulates the effects of insulin on other cells, but efforts to identify a single protein that serves as its receptor have failed. What is more, analyses of the recently completed human genome se-

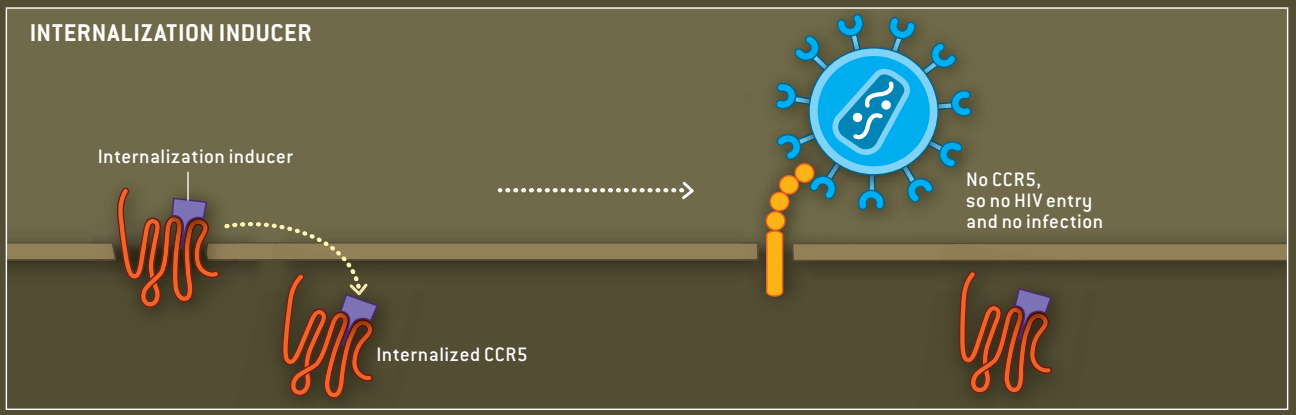
A SCENARIO FOR HIV TREATMENT

At least two new kinds of drugs aimed at CCR5, a G-protein coupled receptor, could one day fight HIV. This virus infects helper T cells of the immune system (*top*). To enter the cells, HIV first adheres to a molecule called CD4 (*1*), an attachment that facilitates the binding of a viral protein (gp120) to CCR5 (*2*). This connection induces the cells to take up the attached virus (*3*), enabling the pathogen to release its genes and convert the infected cell into a virus-making machine.

Allosteric modulators that change the shape of CCR5, making it unrecognizable to gp120 (*top left panel*), are already in clinical trials. Unfortunately, though, viral mutations could eventually alter gp120 so that it became able to attach to the allosterically altered CCR5 and enter T cells (*top right panel*). But an internalization inducer could potentially remove CCR5 from the cell surface (*bottom panel*), making it unavailable even to the mutated virus.







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SOME EARLY PROSPECTS FOR NEW DRUGS

For the most part, investigators are only beginning to devise drugs that influence GPCRs in new ways. But many such agents can be expected to enter pharmaceutical pipelines in the years ahead.

DISORDER	DRUG TYPE	DRUG NAME (MAKER)	TARGET GPCR	STAGE OF DEVELOPMENT
HIV infection	Allosteric modulator	Aplaviroc (GlaxoSmithKline); Vicriviroc (Schering-Plough); UK-427, 857 (Pfizer)	CCR5 (binding by HIV helps the virus enter cells)	All are in phase II or III human trials (early or advanced tests of efficacy)
	Allosteric modulator	AMD3100 (AnorMED)	CXCR4 (this receptor, too, can help HIV enter cells)	In phase III human trials
	Internalization inducer	PSC-RANTES (several institutions)	CCR5	Theoretical
Diabetes	Binder of a receptor formed by two molecules	Symlin (Amylin)	Complex consisting of a protein called RAMP and the GPCR for calcitonin (a thyroid hormone)	Gained U.S. approval in March 2005
Obesity	Inverse agonist	None yet	Constitutively active ghrelin receptor in central nervous system	Theoretical
	Inverse agonist	None yet	Constitutively active histamine H_3 receptor in central nervous system	Theoretical
Cancer	Inverse agonist	None yet	Various constitutively active GPCRs	Theoretical

quence indicate that no gene for such a receptor exists. On the other hand, a complex consisting of the GPCR for the thyroid hormone calcitonin plus a nonreceptor protein called RAMP (receptor activity–modifying protein) responds strongly and selectively to amylin. Apparently RAMP makes the calcitonin receptor "multilingual"—that is, the receptor is reactive to calcitonin if cells lack RAMP, but it is sensitive to amylin if cells contain RAMP.

A different co-protein, called RCP (receptor component protein), induces the calcitonin receptor to obey signals from yet another substance—CGRP (calcitonin-gene-related peptide), a small protein that is the most potent known dilator of blood vessels. This conversion becomes valuable during pregnancy, when blood levels of the dilating peptide soar and RCP levels rise in the uterine wall. As RCP concentrations increase, so do the numbers of calcitonin receptors that become sensitive to the dilator, a change that enhances the blood supply to tissues important for childbirth.

Because co-proteins affect GPCR activity, they might themselves prove valuable as drug targets. One intriguing target is modulin, a co-protein that binds to receptors for serotonin. In the brain, serotonin is most famous as a mood-enhancing neurotransmitter. (Prozac and related antidepressants work by increasing the brain's serotonin levels.) Outside the brain, it acts on the intestines and blood vessels. Perhaps unsurprisingly, serotonin receptors have numerous subtypes, and modulin further tunes the effects of serotonin on particular cells by altering a subtype's sensitivity to it. A drug that mimicked or inhibited modulin, then, could in theory increase or decrease the responsiveness of specific serotonin receptors on specific cell types and might thereby be beneficial in realms ranging from schizophrenia to gastrointestinal function.

Researchers estimate that of the estimated 650 human GPCR genes, about 330 might be blueprints for receptors well worth targeting by drugs. In the past, pharmaceutical scientists would have focused strictly on developing old-fashioned inhibitors or agonists aimed at the receptors' active site. But if many GPCRs offer multiple sites of attack, the opportunities for devising new therapies explode. Because it can take 15 or even 20 years to discover a drug, explore its actions, evaluate its safety, and get it to market, detailed forecasts would be premature. Nevertheless, the new insights into how GPCRs are controlled suggest that these old standbys still have exciting tales to tell. SA

MORE TO EXPLORE

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