Redefining Dioxins

Once dreaded as industrial poisons, some of these compounds may prove to be natural—even beneficial

By JANET RALOFF

Within every cell of the body resides a protein known to scientists as the aryl hydrocarbon receptor. Most people, however, know it by its more ominous name: the dioxin receptor.

It's the site to which a number of industrial chemicals or byproducts bind and start a process that can lead to damage. Like a key in a lock, these hydrocarbons slip into the receptor. If the fit is good, they trigger a cascade of gene actions that can disrupt normal development, impair fertility, and cause diseases, perhaps including cancer (SN: 12/9/95, p. 399).

The key that most effectively unlocks this receptor's toxic potential is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It appears to be so potent that even at background levels in the environment it can alter the development of a child's teeth (SN: 2/20/99, p. 119).

Dozens of other pollutants can also turn on this receptor. To distinguish among these compounds, scientists usually describe TCDD as *the* dioxin and all others as functional dioxins. Though direct exposure to some of these others has been linked to IQ deficits (SN: 9/14/96, p. 165), sickness and behavioral problems can show up even in the offspring of heavily exposed people (SN: 11/11/95, p. 310).

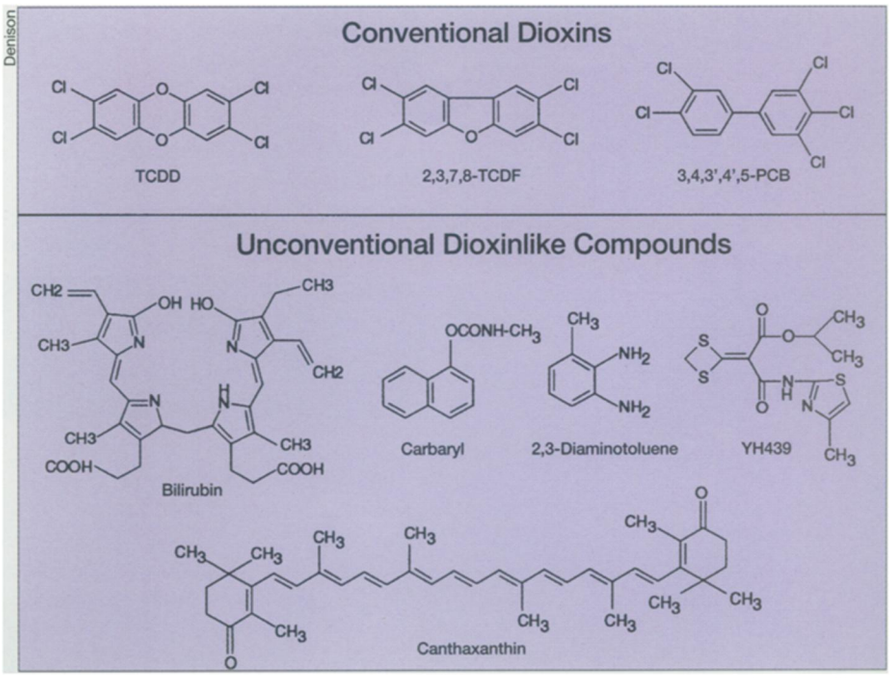
Toxicologists have puzzled over the question of how fish, birds, and mammals evolved a receptor millions of years ago (SN: 5/17/97, p. 306) that could be activated by pollutants that would become ubiquitous only in the 20th century. Furthermore, why would this prescient receptor be distributed throughout the body?

The most reasonable hypothesis was that this lock developed to turn on gene activity in response to some natural keys in the body.

Strong support for this suspicion emerged 3 years ago when Frank J. Gonzalez of the National Cancer Institute and his colleagues bred a strain of mice lacking the gene for the aryl hydrocarbon (Ah) receptor. These so-called knockout mice proved sickly, suggesting that the body needs the Ah receptor for healthy development (SN: 5/6/95, p. 277).

The finding failed, however, to shed light on the dioxin receptor's natural function. Lately, a host of researchers have been focusing on this mystery. While uncovering many tantalizing leads, they all agree on only one thing: The receptor most likely has multiple roles in normal cell functioning, none of which anticipated its response to TCDD.

If any of the potential roles are confirmed, calling this protein the dioxin receptor may malign a molecule critical to good health. What's more, the fact that so many compounds can bind to the receptor may, contrary to conventional wisdom, prove a good thing.



The old idea that a dioxinlike compound has to be a two-ring, planar molecule—like the dioxin, furan, and PCB shown in the top row—is giving way to a more general description. Most known functional dioxins have at least two rings—usually containing nitrogen—and dissolve readily in lipids. However, even this portrayal may need amending. For instance, at least one single-ringed compound, diaminotoluene, can activate the receptor.

Over the past 2 decades, the list of compounds known to turn on the Ah receptor has been growing. While the source and potency of these hydrocarbons can differ widely, all have tended to share a similar molecular structure—two rings and some appendages that lie flat in a common plane.

Last October, toxicologists at the University of California, Davis reported the surprising finding that carbaryl, a widely used insecticide, also activates the Ah receptor (SN: 11/28/98, p. 344). What distinguishes carbaryl is its nontraditional structure, explains Michael S. Denison, who led the research. While it possesses a pair of rings, the side chain that dangles off of it does not lie in the same plane as the rings.

Suspecting that many other dioxinlike compounds might have been missed over the years because they, like carbaryl, don't resemble TCDD, Denison's group has begun a new screening program. The team hopes to uncover natural

keys for the Ah lock. The researchers have selected some 600 prospects—including many of the body's signaling agents, such as prostaglandins, lipids, and neurotransmitters—and have begun running them through bioassays to see whether they fit the Ah receptor.

The effort "has turned up a couple of these [nonclassically shaped] compounds that look quite promising," Denison says.

In the Aug. 18, 1998 BIOCHEMISTRY, he and his coworkers described finding some Ah receptor binding by tryptamine and indole acetic acid, two breakdown products of the ubiquitous amino acid tryptophan. They also reported, in the Sept. 1, 1998 ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, a binding by bilirubin and biliverdin, chemicals that form during the natural destruction of aging red blood cells. However, all four compounds appear to bind too weakly to activate the receptor under normal conditions.

Thomas A. Gasiewicz, a toxicologist at the University of Rochester (N.Y.) Medical Center, notes, however, that these breakdown products "may serve some very important local function at very focal times in a particular tissue's development."

Why did the body develop a lock that many keys can open? The explanation that Denison favors is that the body employs the Ah receptor as the trigger for a general-purpose detoxifying system.

Most compounds that bind to this receptor, he explains, are actually destroyed by the actions they initiate. The gene activity that they trigger produces enzymes that rapidly break down the invading chemical.

"Because we get exposed to so many natural toxicants, I think this turns out to be a beneficial protection system," Denison argues.

Unfortunately, he adds, the system fails when it encounters TCDD because it is so resistant to the hydrocarbon-degrading enzymes whose production is triggered by its binding to the Ah receptor. Not only does it not disappear, but TCDD loiters in the lock, triggering a seemingly endless production of the destructive enzymes.

Christopher A. Bradfield of the University of Wisconsin-Madison's McArdle Laboratory for Cancer Research calls Denison's detoxification explanation for the Ah receptor "a compelling story." At least seven genes that foster the metabolic breakdown of functional dioxins are directly activated via the Ah receptor, he notes. However, he adds, "that doesn't make it the only story."

Work in a number of labs, including Bradfield's, suggests that the Ah-receptor protein traces evolutionarily to sensory systems in bacteria. Independent of any chemical-detoxification role, Bradfield says, the Ah receptor seems capable of turning on signals that can play a critical role in animals.

Lending support to this idea is the Ah receptor's resemblance to some very primitive proteins, maintains J. Clark Lagarias, a plant biochemist at the University of California, Davis. The Ah-receptor protein contains a region characterized by a pattern of repeating amino acids, called the PAS domain. Such a pattern shows up in proteins carrying biochemical signals within organisms from purple bacteria to people.

When this domain encounters its environmental trigger—be it light or some chemical—its shape changes, "setting in motion a cascade of biochemical events" including gene activation, Lagarias explains.

Some light-sensitive proteins with PAS domains help microbes move toward the sunlight they need for energy, he notes. Other PAS-domain proteins signal the presence of toxicants that a bacterium should avoid or foods that it should seek.

Through billions of years of evolution, many of the organisms that use these proteins have become more complex, and so have their locks. With the Ah receptor in animals, Lagarias says, "we're looking at a modern descendant of those bacterial locks." They can now respond not just to outside environmental cues but to internally generated ones as well.

Agneta Rannug of the Karolinska Institute in Stockholm and Ulf Rannug at the University of Stockholm report that they have uncovered one such signaling role that occurs in animals, including humans.

Denison's work focused on the Ah receptor's ability to trigger any of several genes, such as *CYP 1A1*, that make enzymes to begin detoxifying poisons. The Rannugs note that shining light on skin cells grown in the test tube also increases the activity of *CYP 1A1*.

"What has never been explained," Agneta Rannug says, is why light should have this effect. The Rannugs and their colleagues now suggest that this light exposure breaks tryptophan down into its metabolites, which then "can spread throughout the body," triggering the Ah receptor—and, eventually, the *CYP 1A1* gene's production of those enzymes.

At an international dioxin conference in Stockholm last year, the Rannugs and Yu-Dan Wei of the University of Stockholm unveiled test-tube studies with human cells. Their data indicate that some metabolites of tryptophan, different from those that Denison has been studying, are potent activators of the Ah receptor. The Rannugs believe that these chemicals "belong to a new class of signaling substances that may function as chemical messengers of light," compounds that may even play a role in the body's biological clock.

Pharmacologist Stephen Safe of Texas A&M University in College Station has identified another possible role for the Ah receptor.

The presence of a dioxinlike key has always been thought necessary to activate the receptor. However, Safe's studies now indicate that when the Ah-receptor lock is keyless, it will link up with another protein, known as Arnt. In partnership, these

Tapping dioxins as anticancer drugs

Researchers have linked diets rich in brassicas—such as cabbage, broccoli, and brussels sprouts—to a reduced risk of cancer (SN: 9/20/97, p. 183). During their digestion in the gut, these foods produce indole-3 carbinol (I3C), a compound that may prove useful in fighting cancers (SN: 7/3/93, p. 10).

Seven years ago, Christopher A. Bradfield, then at Northwestern University, showed that I3C activates the Ah receptor.

"The gastrointestinal tract is a little chemical manufacturing plant, where microbes are pumping out products like crazy," Bradfield observes. It makes sense, he says, that the gut would have proteins on hand to detoxify and dispose of any druglike chemicals that intestinal bacteria might brew up from the foods people eat.

Stephen Safe of Texas A&M University in College Station has been probing I3C's anticancer activity. In the gut, pairs of I3C molecules form a stable structure that can bind to the Ah receptor. "When we give this compound to rats with small mammary tumors," he says, "the tumors stop growing."

In the October 1998 CARCINOGENESIS, he reports evidence that this is an Ah-receptor–mediated process. "Now we're developing a whole series of analogs of this vegetable-derived Ah-receptor activator for the treatment of breast cancer," he says.

So far, Safe has patented two such chemicals, which he and his colleagues are testing in animals. "Both inhibit tumor growth and appear nontoxic," he says. The new chemicals have no estrogenlike activity, he adds, an important trait for drugs targeted against cancers that use estrogen to fuel their growth. —*J. Raloff*

A breakdown product of broccoli and related vegetables is serving as a model for novel anticancer drugs that may work through the Ah receptor.

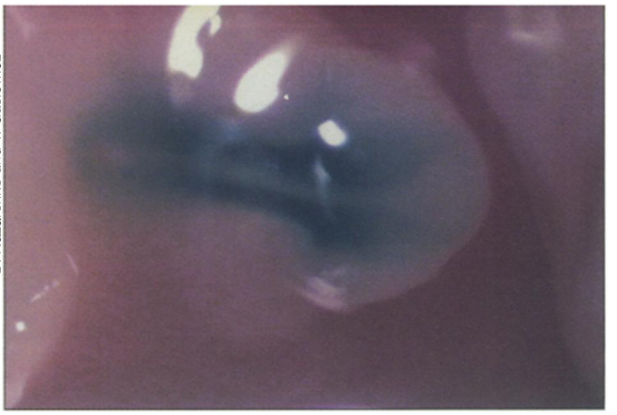
proteins can then "act to regulate expression of some genes" by binding to specific parts of their DNA, Safe has found.

In the June 1998 NUCLEIC ACIDS RESEARCH, Safe and his colleagues showed that cellular production of an enzyme known as cathepsin-D can triple or quadruple under the influence of such a partnership. Once some compound binds to the Ah receptor, this enhanced gene expression drops. This suggests, he told SCIENCE NEWS, "that there may be a natural function of the [keyless] Ah-receptor complex in regulating normal gene expression in cells."

Gasiewicz cautions, however, that the Ah receptor's dark sides shouldn't be forgotten. For example, he says that his recent work suggests that the Ah receptor may serve as "one of the body's master switches" for turning on genes "that transform compounds in cigarette smoke into more toxic chemicals."

He incubated human cells with extracts of cigarette smoke and then scanned the cells for fragments called micronuclei, which are evidence of precancerous genetic changes. Cells engineered to lack the Ah receptor produced few such micronuclei, while those with the receptor made many.

Then, his team exposed mice—both normal ones and knockout mice lacking the Ah receptor—to cigarette smoke. In the November 1998 CARCINOGENESIS, his team reported that the normal mice developed many of the micronuclei, indicating gene damage, while the knockouts exhibited none. Exposure to TCDD further increased the number of micronuclei in normal animals exposed to smoke. Says Gasiewicz, "They got a double whammy."



D. Nazarenko and T. Gasiewicz

Scientists at the University of Rochester have developed a blue staining technique to highlight cells whose Ah receptors have been turned on. Here, the triggered cells reside in the genitalia of a fetal mouse whose mother was given a nontoxic dose of the dioxin TCDD 24 hours earlier, 1 week before birth. Researchers have known that a developing rodent's genitals are a target for TCDD toxicity. This staining is the first step in identifying which cells are affected and when.

Taken together, these studies are teasing out new roles—and respect—for the long-dreaded Ah receptor. The most unexpected dividends of Ah-receptor understanding may emerge in medicine. Delineating what compounds trigger the receptor, and when, may lead to more effective cancer therapies (see sidebar). Gasiewicz's team is also investigating the prospect of engineering drugs to block Ah-receptor activity. They might limit toxicity in smokers or people accidentally exposed to TCDD.

"We might even be able to achieve some of this protection naturally" by, for instance, eating more vegetables like broccoli, Gasiewicz says. "But we can't hope to do that without a better understanding of this receptor."

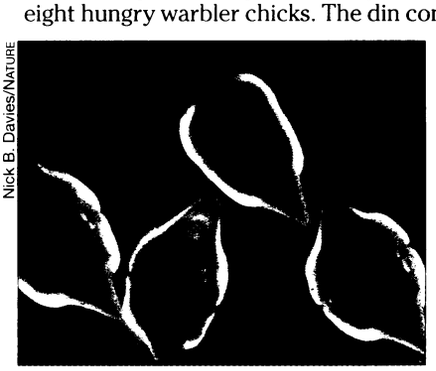
Biology

Cuckoos beg doggedly to trick hosts

Cuckoos are practiced in the art of deception. Once a clandestinely laid cuckoo egg hatches in a reed warbler nest, the chick evicts its native nestmates. The parents, as if feeding their own feathers and blood, tend to the baby cuckoo until it grows larger than both of them together and finally flies away.

Researchers have decoded how one cuckoo elicits the same feeding behavior as a nestful of hatchlings. Rebecca M. Kilner of the University of Cambridge in England and her colleagues determined that baby reed warblers signal the degree of their hunger in two ways—wide-open beaks and persistent calling. Parent warblers, they found, respond to the combination of these signals and feed their young accordingly.

The researchers then examined how the cuckoo manages "a bit of bait and switch," as Douglas W. Mock of the University of Oklahoma in Norman says in a commentary accompanying the Feb. 25 NATURE report. The cuckoo, with its single gaping beak, cannot duplicate the visual pull of a throng of baby warbler beaks, so it tugs at the parents' other heartstring. The baby cuckoo calls so persistently and rapidly that it sounds like a brood of eight hungry warbler chicks. The din compensates for the cuckoo's less stimulating small gape, and the parent warblers feed it generously. —*L.H.*



Nick B. Davies/NATURE

Because the common cuckoo can't display a nestful of gaping reed warbler beaks (left), it begs by imitating a chorus of chicks.

Elephants can die from herpes viruses

Two herpes viruses new to science can attack and kill elephants, pathologists report.

These are the first herpes viruses found in elephants, says codiscoverer Laura K. Richman of the Johns Hopkins Medical Institutions in Baltimore. The viruses hold special interest because they jump from one kind of elephant to another in zoos that are trying to protect the endangered species.

Many animals, from people to fish, suffer from cold sores and other miseries of herpes infections. The discovery of elephant herpes, described in a Feb. 19 SCIENCE paper, is a whodunit solved by Richman, Gary S. Hayward from Johns Hopkins, and Richard J. Montali from the National Zoo in Washington, D.C. They set out in 1995 to find the killer of Kumari, the first apolitical elephant born inside Washington's Beltway.

The 16-month-old died after a mysterious illness that took away her appetite, left her listless, and made her tongue turn purple. Her autopsy showed massive internal hemorrhaging. Microscopic examination of tissue and DNA analysis revealed a previously unknown herpes virus. The researchers found the same virus in preserved tissues from seven other Asian elephants that had died mysteriously in North American zoos.

The same infection also turned up in African elephants in zoos. Yet this closely related species seemed to suffer nothing more serious than skin nodules and small vaginal lesions. A normally minor African virus could be turning lethal when it jumps to a different species, Richman warns. Identifying the infection early, in part by the purple tongue, allowed zookeepers in Missouri to save one young elephant with massive doses of the human herpes drug famciclovir.

The researchers have also discovered a related herpes virus that killed a young African elephant. So far, the researchers have not found a clue to its source. —*S.M.*