NMDA Receptor Cloned—Twice!

Two groups claim to have cloned a long-sought-after receptor on brain cells—but only one of them can be right

MOLECULAR BIOLOGISTS SOMETIMES LIKEN the competition between labs to clone a specific gene to a horserace. By that analogy, there's just been an extraordinary finish in the Kentucky Derby of gene clonings—indeed, a photo-finish with a wild twist: one of the horses is likely to have fouled down the stretch.

In the 7 November issue of *Nature*, Shigetada Nakanishi and his colleagues at Kyoto University in Japan and, separately, Elias Michaelis and his co-workers at the University of Kansas at Lawrence announce that they have independently cloned the NMDA receptor. This is not your gene-of-the-week trifecta: the NMDA receptor is a cell-surface molecule that may play a key role in learning and memory. Not only that, it has been implicated in neurodegeneration and stroke-related brain cell death. That drove the stakes of what had been a frustrating half-dozen-year marathon through the roof: pharmaceutical companies have been pouring millions into finding drugs that alter the receptor's activity. Ray Dingledine, a University of North Carolina at Chapel Hill neurobiologist who has been one of the questers, puts it bluntly: "The NMDA receptor is the granddaddy of [its class of receptors], the one that everyone's been going for."

But don't assume it's time to hand out the big old silver soup tureen (or to clone it so two teams can put it up on their trophy shelf). In a bizarre twist, the judges of this photo-finish (the scientific community) are going to have to spend quite a time going over the photos. The problem is: A cursory glance at the pix suggests that the horses were running on different tracks! The two groups, it seems, have come up with entirely different genes.

The protein found by Nakanishi and colleagues in Kyoto is twice as large as the one found by Michaelis and co-workers in Lawrence. What's more, the gene cloned by the Kansas group encodes a protein that functions in a complex of four. The gene found by the Japanese group, in contrast, codes for a protein that contains all of the receptor's activities in a single molecule.

The crucial question, of course, is who is right. At this early stage, the smart money seems to be on the Japanese. "Nakanishi's [cloning method] generated a clone that had virtually all of the properties of an NMDA receptor. Michaelis found something that binds glutamate," says neurophysiologist Mark Mayer of the National Institutes of Health, whose research centers on the pharmacology of the receptor. Moreover, there's a track record to be taken into account: Several scientists point out that the methods used by the Michaelis group have in the past led other researchers to misidentify receptor proteins. "There have been instances where proteins identified by [the approach used by Michaelis] have turned out to be artifactual," says Charles Stevens, a neurophysiologist at the Salk Institute in La Jolla, California. He adds: "The problem is often with contaminants. One might worry about that here."

"The NMDA receptor is the granddaddy of [its class of receptors]."

—Ray Dingledine

As if all this didn't make the NMDA derby intriguing enough, even if a winner is declared, the victorious team will have questions to answer. Both models will have to explain curious, intriguing—almost paradoxical—features of the receptor. NMDA stands for N-methyl-D-aspartate, an artificial compound that selectively activates one of the several types of brain-cell receptors that respond to the amino acid glutamate, which serves as an excitatory neurotransmitter.

The NMDA receptor doesn't behave like the two other broad types of glutamate receptors in the brain. One class, the kainate receptor, admits ions through a channel in the receptor protein, triggering an electrical impulse in the target cell. The binding of glutamate to the other type (the metabotropic glutamate receptor, a gene for which was cloned earlier this year by Nakanishi's group) has an entirely different effect, triggering a chemical cascade in the interior of the target cell that leads to, say, gene expression or neurotransmitter synthesis. Most receptors in the brain carry out one or the other of these functions exclusively—but NMDA is an exception. "NMDA receptors represent a funny middle ground," says Stevens. "They do both."

And that unusual combination of qualities probably plays a role in the widespread activity of the receptor in the brain. The consensus in the field is that NMDA receptors are central to long-term potentiation (LTP), the persistent strengthening of neuronal connections that probably underlies learning and memory.

In addition, when brain tissue is damaged by a stroke or heart attack the cause may be the overexcitement of cells carrying NMDA receptors ("excitotoxicity" as it is called). Rather than dying from oxygen deprivation, as is commonly assumed, says Stevens, "maybe 80% of cells die by exciting themselves to death. After excitotoxic cell death [in the hippocampus], people can't learn new things, but they can remember things already stored in memory. If we knew the structure of the receptor, we could develop better drugs," he says. Those drugs, by blocking the receptor, might well save the brain tissue of stroke or heart attack victims.

All of which clearly makes it that much more important to know which group has actually found the NMDA receptor. One clue that may help the judges decide who belongs in the winner's circle involves the highly disparate approaches and findings of the two teams. The basis of the Kansas team's approach was an indication earlier this year from the lab of Mordichai Sokolovsky at Tel Aviv University that the NMDA receptor might include several different proteins joined in a complex. "The idea had been percolating that perhaps the receptor was made up of several different subunits and unless you cloned out all of them, you wouldn't get receptor activity," says Mayer.

Following the Sokolovsky lead, the Michaelis group purified four different proteins that they are convinced make up the receptor complex. The gene they cloned encodes the largest of the four. Their reasons for thinking they've got the right molecule include the observation that when they put the purified components in artificial membranes, they can simulate full receptor activity. The reconstituted receptor is responsive to all the drugs that the native receptor responds to and is unresponsive to the drugs the native receptor fails to respond to. In addition, says Michaelis, "we see that the gene is expressed in those regions of the brain where NMDA receptors are particularly abundant—the hippocampus and the cerebellum."

One thing that troubles some workers about the Michaelis group's finding is that the gene they've cloned doesn't seem to be related to other glutamate receptors. "The

sequence of this gene doesn't match that of any of the previously described glutamate receptors," Michaelis concedes. Nevertheless, he argues that the activity of the encoded protein is consistent with what is known about the receptor they're seeking. "At this stage, I think our receptor matches quite well the known literature on NMDA receptors."

Precisely the same claim, however, is made by Nakanishi and his colleagues. "Our receptor has the function of the NMDA receptor. I am satisfied that our results are consistent with the predictions for this receptor," he says. When Nakanishi put his receptor protein through its paces, it performed as expected: It allowed the passage of calcium ions, it responded to the same drugs as the native receptor and was unresponsive to those that the native receptor would not respond to.

Furthermore, the structure of the single protein found by the Kyoto group is similar to other glutamate receptor proteins—including the kainate receptors. Like those other known receptors, says Nakanishi, the protein they have found has four segments crossing the membrane, and the portion of the molecule that sticks out of the cell is especially large.

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—Mark Mayer

For Mayer and others who think the Japanese are running on the right track, the clincher is that the receptor found by the Kyoto team is co-activated by the amino acid glycine, which, says Mayer, is a "hallmark of the NMDA receptor. We do know from Nakanishi's work that a single receptor subunit when combined with more of the same will make a functional receptor."

But the final determination of which group really has the receptor can be worked out only by performing more functional studies on protein chemistry. That work is now under way in the Nakanishi and Michaelis laboratories, and, given the recent publication, will likely be pursued in many other labs as well. "When these sequences come out, people will try to reclone it instantly," says Stevens. And the biochemical work done in those labs on the two clones will provide the late result in this longest-running of gene-cloning horseraces.■ MICHELLE HOFFMAN

Ancient Rocks, Rhythms in Mud, a Tipsy Venus

The Geological Society of America's annual meeting, held 21 through 24 October in San Diego, drew 6000 geologists and spanned planets' worth of news—the earliest plate tectonics on Earth, the latest revision of the geologic timescale, the gregarious volcanoes of Venus. Here are findings that captivated all who heard of them.

Pushing Plate Tectonics Back a Billion Years

According to the conventional wisdom in geology, plate tectonics—the jostling of great sheets of rock that now shapes much of the earth's surface—didn't get started until at least 2 billion years after the planet's formation. Rocks older than that just looked too different from those being produced by plate tectonics today. But at the meeting a group of Japanese scientists argued that plate tectonics was already under way almost 4 billion years ago, less than a billion years after the planet's formation. The evidence, they say—the debris of an ancient continental collision—is there for all to see on the west coast of Greenland.

That's going to be a lot for some geologists to swallow. In the 20 years since earth scientists convinced themselves that the planet's surface is a jigsaw puzzle of moving plates, the agreed starting point for the process has been pushed back—from 250 million years ago to perhaps 2.5 billion years ago—as geologists grow more adept at recognizing the signature of plate tectonics despite the ravages of time (*Science*, 20 December 1985, p. 1364).

Some geologists said they could see plate tectonics at work in even older rocks, from the Archean eon, but many researchers could not and have refused to go that far. After all, theorists had an explanation for the absence of plate tectonics before 2.5 billion years ago. At the end of their geologic lifetimes, plates sink back into the planet's interior; before 2.5 billion years ago, said the theorists, Earth's surface layers were simply too hot and buoyant to do so.

"We needed direct evidence of plate tectonics" to resolve any doubts, says Shigenori Maruyama of the University of Japan at Komaba, "and we found it at Isua." What Maruyama and his colleagues thought they saw among the 3.8-billion-year-old rocks at that well-studied field site near the southwest coast of Greenland were abundant signs of ocean crust that had been formed and transported by plate tectonic processes. Although metamorphosed by burial deep beneath the surface in the interim, the classic components of the upper ocean crust were recognizable, according to Maruyama. From the top down, there were sediments, pillow-shaped mounds of lava that only form under water, and the silica-poor rocks that typically form in the magma chambers beneath the mid-ocean ridges of the recent past. These rocks thus gave every sign of having formed at an ancient mid-ocean ridge, says Maruyama; plate motions must then have carried the newborn crust away until it collided with an ancient continent.

That's just the process that has built much of the crust of modern-day island arcs like Japan. Indeed, Maruyama credits the discovery to his group's familiarity with such accreted ocean crust in Japan. Because they are younger, those rocks served as a good training ground for recognizing similarly produced rocks in Greenland despite 4 billion years of change, he says.

"This is a very innovative and exciting interpretation," says geologist Samuel Bowring of the Massachusetts Institute of Technology. Given earlier geologic, geochemical, and geochronological evidence, Bowring was already inclined to push plate tectonics back into the Archean. In the light of the new results, he says, "the burden of proof should be with those who say things were different in the Archean." The rocks of Isua will no doubt be at the center of that debate.

Ocean Mud Pins Down a Million Years of Time

The pace of the past million years of earth history just slowed down a little, thanks in large part to the persistence of a handful of paleoceanographers. Based on a record of Earth's clock-like nodding and wobbling preserved in deep-sea sediments, they have been arguing that the traditional date for a key event in the past million years, derived from the steady decay of radioactive isotopes, is off by as much as 50,000 years. Now, new radiometric dates presented at