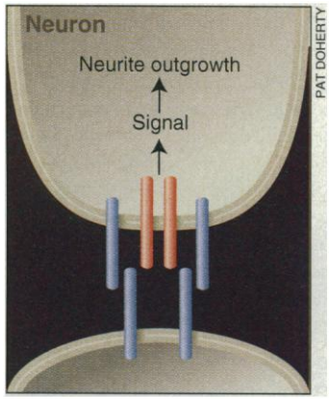
CELL BIOLOGY

Neuronal Adhesion Molecules Signal Through FGF Receptor

Every part of embryonic development is complex, but the task faced by a developing neuron may be the most daunting. A young nerve cell must send out projections that traverse great distances, often many inches—or even feet—through an obstacle course of other cells, and then make a functional contact with exactly the right target cells. The failure to accomplish this feat carries a steep price: The neuron dies. To help nerve cells avoid that fate, the neuronal projections have guides on their journey. Among them are the cell adhesion molecules (CAMs). These proteins on the surface of the neuron link up with similar molecules on other cells or on the proteinaceous extracellular matrix, and, by making the right connections, serve as a kind of railway switching system that enables the neuron to reach the right destination.



Getting together. Contact between a neuronal CAM *(blue bars)* and its counterpart on another cell may lead to activation of the FGF receptor *(red bars)*.

Just how the CAMs play that signalbox role has been somewhat mysterious, however. Until about 6 years ago, neurobiologists thought CAMs offer only passive structural support for growing neurons, while other molecules, such as growth factors, stimulate the outgrowth of neuronal projections (neurites). But recent evidence suggests that the CAMs play a more active role. The question is how. As developmental and cell biologist Jean Paul Thiery of the CNRS-Ecole Normale Supérieure in Paris notes, CAMs "are not intrinsically endowed" with the ability to transmit growth-stimulatory or other signals to the cell interior where they will be acted upon.

Now, new research is providing that link, joining the CAMs to growth-control signaling pathways in different cell types. Some of the most advanced of this work deals with neurons and comes from Pat Doherty and Frank Walsh's group at the United Medical and Dental School, Guy's Hospital, London. They've shown that certain CAMs activate the receptor for fibroblast growth factor (FGF), which in turn sets off internal cellular events needed for neurite outgrowth. Although there's growing evidence that CAM-initiated signaling pathways interact with pathways triggered by other molecules, this is the first evidence that CAMs can signal by "hijacking" the receptor for another molecule. "These are exciting and important observations," says cell biologist Alan Horwitz at the University of Illinois, Urbana-Champaign. "They begin to answer some old questions, such as why is adhesion required for many cellular processes, like [cell] survival and proliferation."

What's more, even though the CAMs normally do their job by modulating cell adhesion, the Doherty-Walsh team found that adhesion isn't required to stimulate neurite outgrowth. One CAM that they rendered nonadhesive still stimulated growth of neurites. This raises the possibility that such altered adhesion molecules might be used in therapies for stimulating nerve regrowth after damage. Any such application lies far in the future, however. At the moment, investigators are still puzzling through the new evidence about how CAMs do their regular jobs.

An early clue that some CAMs might associate with the FGF receptor came 3 years ago from Orest Blaschuk's group at McGill University and Royal Victoria Hospital in Montreal, Canada, although no one noticed it at the time. These researchers were studying a group of CAMs known as cadherins, located on the outer membranes of cells, including neurons. Cells bearing identical cadherins bind to one another through the contacts made by these CAMs, and Blaschuk and his colleagues had identified a specific amino acid sequence (His-Ala-Val) that seemed to be important for the molecules' interactions.

Then in 1992, the group showed that the same amino acid "motif" is also present in the part of the FGF receptor that extends outside the cell. This finding provided a hint that cadherins might also be able to interact with the FGF receptor. But at the time, Doherty recalls, "it was overlooked by other groups in the field." Even he didn't spot the significance, he admits, until 1993 when the Guy's Hospital group was studying the properties of two other important neural cell adhesion molecules: N-CAM and L1.

The researchers had been analyzing a region in the extracellular portion of N-CAM that is crucial for neurite outgrowth, and on running the specific motif through a protein sequence database, Doherty found that the best match turned up in an extracellular sequence of the FGF receptor. On closer inspection, they found that the same portion of the FGF receptor also contains the His-Ala-Val cadherin motif and another short amino acid sequence identical to one in L1, bringing the total of CAM-related sequences to three, all in a single 20–amino acid region of the FGF receptor that Doherty dubbed the "CAM homology domain."

This observation happened to connect with some other results from the same group—much as one CAM binds to another. With Walsh, Doherty had been characterizing the intracellular signaling events underlying the stimulation of neurite outgrowth by CAMs. They had noted that the sequence of events begins with the activity of a regulatory enzyme called a tyrosine kinase, which adds phosphate groups to proteins. CAMs don't have tyrosine kinase activity, but the FGF receptor does, and Doherty says, "The fact that the FGF receptor contained this CAM homology domain really excited us," because it suggested that CAMs might be acting by taking over the FGF receptor.

After this "aha" moment, the researchers began testing whether FGF receptor function is required for CAM responses, and now they've got several lines of evidence to show that it is. Normally, for example, neurite outgrowth can be stimulated by culturing neurons with fibroblast cells that have been engineered to make any one of three CAMs—N-CAM, L1, or N-cadherin. But in the September 1994 issue of *Neuron*, the group reported that they could block the response with antibodies that bind to the FGF receptor's CAM homology domain, apparently preventing the CAMs' interaction with the receptor.

The importance of the FGF receptor for stimulating neurite growth was brought home further by experiments in which Doherty and Walsh made a soluble form of L1 CAM by cutting off the portion that would normally be embedded in the cell membrane. As described in the January 1995 issue of *Neuron*, they found that this protein, added to the culture medium, also stimulates neurite outgrowth, even though it does not provide adhesive support to the neuron. This work shows, Doherty says, that "signaling is more important than structural adhesion per se." That's also encouraging, he notes, because it raises the possibility of using such soluble CAMs therapeutically, to stimulate nerve regrowth after damage, although much more work will be needed to determine if that's feasible.

Meanwhile, because all the work so far has been done with cultured neurons, the researchers want to confirm that the FGF

receptor is playing the proposed role in CAM responses in the brains of living animals. Also unclear is exactly how the CAMs activate the FGF receptor. Although other scenarios haven't been ruled out, Walsh and Doherty think that the current evidence favors the idea that stimulation of neurite outgrowth begins with a CAM on a growing neuron contacting a CAM on a neighboring cell. This recognition event then leads the CAM on the neuron to nudge up against an FGF receptor in the same membrane, activating the receptor tyrosine kinase and setting off a pathway that ultimately produces neurite growth.

While Doherty and Walsh were the first to suggest a direct molecular interaction between adhesion molecules and a growth factor receptor, other researchers are also pursuing the interactions between CAMs and cellular growth pathways. But in these cases the adhesion molecules generally don't interact directly with the growth factor receptors themselves. Take the integrins, which attach neurons and numerous other cell types to the proteins of the extracellular matrix. Integrins appear to interact with pathways activated by platelet-derived growth factor and insulin, but in this case the two signals appear to cross paths inside the cell, downstream of the growth factor receptors.

These findings are intriguing, says cell biologist Erkki Ruoslahti of the La Jolla Cancer Research Foundation in California, one of the researchers doing the integrin work, because it suggests there are many possible levels of interaction between CAMs and growth signaling pathways. "We know that FGF receptors do not cooperate with [the] integrin [that interacts with insulin signaling]. Perhaps every adhesion receptor has its own growth factor receptor partner!" Indeed, Thiery predicts that the emerging story of CAM action will force a re-evaluation of the molecules' role in development and other cell activities. "These adhesion molecules," he says, "are perhaps smarter than we thought at first."

–Claire O'Brien

Claire O'Brien is a science writer in Cambridge, U.K.

ENDANGERED SPECIES

Ivory Identity Crisis Still Unsolved

How can you tell one piece of ivory from another? That question vexes would-be ivory traders in Africa, where a bloody decade of poaching in the 1980s decimated many elephant herds, especially in east Africa. In response to the slaughter, in 1989 the Convention on International Trade in Endangered Species (CITES) banned international trading of ivory. East African nations support the ban, which has had the desired effect: The price of ivory has plummeted, and poaching pressure has plunged along with it. But in southern Africa, wildlife managers are killing elephants to keep their numbers in check and are watching stockpiles of ivory grow. As early as 1992, these nations pushed to reopen the ivory trade. But opening the trade would require a reliable method to distinguish legitimate sources from black-market ivory taken from poached animals.

Back in 1990, it seemed that geochemistry could come to the rescue. Two pilot studies published in *Nature* that year suggested that the isotopic composition of ivory could provide a cheap and easy way to pinpoint its source. Elephants pick up the isotopic imprint of their habitat, and the pilot studies suggested that each locality sampled had a characteristic isotopic "fingerprint." But on page 1340 geochemist Paul Koch of Princeton University and his colleagues report new results that cast doubt on the usefulness of isotopes for this work.

They found that when habitats change—as is happening in many parts of Africa—isotope values in elephants change, too. As a result, animals from a single locality showed a wide range of isotope values rather than a unique signature. "The study shows that this technique doesn't work to source ivory," says Andrew Dobson, an elephant ecologist at Princeton who is familiar with the work. "There's just too much variability within one locality." Although the authors of the original studies aren't ready to give up on their method, the new results weaken the case for opening any trade in ivory, says Dobson.

Like the pilot studies, the new work probes the carbon, nitrogen, and strontium isotopes in elephant bone and teeth. (Koch wasn't allowed to analyze tusk because importing tusk samples would violate U.S. law, according to the U.S. Fish and Wildlife Service.) But while the earlier papers sampled localities around Africa, Koch focused on one well-studied area—Amboseli National Park in Kenya. The isotopic values of Amboseli elephants overlapped somewhat with those measured in animals elsewhere in Africa. The carbon isotopes from Amboseli animals were particularly variable—"almost useless for forensics," Koch says.

KOCH *ET AL.*

Heavy meal. A grass diet gives elephants a higher ^{13}C to ^{12}C ratio than browse does.

In Amboseli, this variability reflects a change in habitat caused partly by the animals themselves. Elephants eat trees and shrubs (browse), as well as grass. During the past 15 years or so, the animals have concentrated in Amboseli, eaten the available browse, and the park's grasslands have expanded. As a result, the elephants' diet has become richer in grass. Because grasses contain a higher proportion of ^{13}C than browse, this shift showed up in the isotopic ratios. Elephants who died in the 1970s, for example, had lower ratios of ^{13}C to ^{12}C, indicating a diet rich in browse, than did elephants who died in the 1980s. Also, microsamples from molars of different animals varied widely as a result of individual behavior. For example, two elephants called Ruth and Zach managed to find trees and shrubs in the mid-1980s, apparently by sneaking out of the park at night. The bottom line, says Koch, is that the isotopic technique is problematic for tracking ivory, although it provides information on elephants' eating habits and migrations.

But those who pioneered the method are more optimistic about its potential. John Vogel of the Council for Scientific and Industrial Research in Pretoria, author of one of the previous studies, agrees that the variability in carbon isotopes makes them less useful, but maintains that ivory sources can still be identified by a mix of isotopes. The author of the other pilot study, Nikolaas van der Merwe of Harvard University, now at the University of Capetown in South Africa, adds that the method could distinguish among a short list of suspected sources.

It may be possible to run a suite of isotopes to distinguish elephant populations, agrees Koch—but it would require a detailed isotopic map of Africa, which is a daunting project in its own right, plus several hundred dollars' worth of tests on each tusk. Furthermore, he notes that in his study, isotope ratios in molars were more variable than in bone. Tusks grow faster than teeth and are likely to be even more variable, he says. The true test, using tusk microsamples from Amboseli and elsewhere, has yet to be done. For now, says Koch, the message is that the technology is not yet ready to support a resumption of ivory trade.

–Elizabeth Culotta