On the Right Track to the NGF Receptor

The trk *oncogene may provide the missing link that will help researchers understand how nerve growth factor transmits its signals into nerve cells*

A YEAR AGO, NO ONE WOULD HAVE THOUGHT Luis Parada and Moses Chao were chipping away at two sides of the same problem. Chao, a neuroscientist at Cornell University Medical College in New York, was trying to learn how the receptor for nerve growth factor (NGF) transmits NGF's message inside nerve cells. He and others in the field were stumped. They had isolated an apparent receptor for the growth factor, but cells with this receptor alone didn't respond to NGF. There seemed to be something missing—perhaps another component that was needed for it to function fully.

Meanwhile, Chao's friend Parada, of the National Cancer Institute's Frederick Research Center, was studying the role of an oncogene called *trk* (pronounced "track"). Since *trk* was originally discovered in a colon cancer, Parada had no reason to expect it would have anything to do with NGF—until he found that the gene was active in some of the same neurons that respond to NGF. Still, no one was jumping to any conclusions that it had a role in the growth factor's action. But it now appears that it does.

On page 554, Parada and his colleague David Kaplan together with Chao and his collaborator Barbara Hempstead report that Trk, the protein encoded by the *trk* gene, may be the long-sought missing component of the NGF receptor. And Mariano Barbacid and his colleagues at Bristol-Myers Squibb Pharmaceutical Research Institute in Princeton, New Jersey, describe similar results in the 5 April issue of *Cell*. The news—which circulated as rumor months before the papers appeared—has created a flurry of excitement in both the neural development and oncogene fields.

"It's a very important observation," says NGF researcher Eugene Johnson of Washington University. "A lack of understanding of the structural basis of the [functional] NGF receptor has been holding this field back for years."

Indeed the discovery may clarify the workings not only of NGF, but also of several other factors that, like NGF, aid nerve cell survival. Knowing how the receptors for these factors function is likely to lead to an understanding of the metabolic pathways important for neuronal survival—pathways that may break down in neurodegenerative diseases such as Huntington's and Alzheimer's, and whose understanding might even lead to new treatment strategies.

The story of the *trk* gene starts back in 1985 when it was cloned from a human colon tumor by Dionisio Martin-Zanca, a postdoc with Barbacid who was then at the Frederick research facility. Even then there was evidence that the Trk protein was a receptor. The *trk* gene sequence revealed that the Trk structure resembles those of known members of the tyrosine kinase receptor family. When triggered, these receptors add phosphate to the amino acid tyrosine on proteins inside the cell, an action that leads to various cellular responses. The molecules that trigger these receptors, known as ligands, have been found for some of the tyrosine kinase receptors; they include agents such as epidermal growth factor that stimulate cell growth. But when *trk* was discovered, its ligand was a mystery.

Nor were Martin-Zanca and Barbacid able to locate the cells where the *trk* gene was active normally. In the cancer cells, it had undergone a mutation that had apparently caused its abnormal activation, thereby contributing to the cancer development. But *trk*, like other oncogenes, presumably had a normal function as well. The gene did not appear to be turned on in any of the other human and animal tissues examined, however.

And there matters stood until 1987 when Luis Parada arrived at Frederick and asked Martin-Zanca to join him in cloning the mouse *trk* gene. Parada hoped the mouse gene would be more useful in the search for *trk* expression than the human gene had been. And he was right. When Parada and Martin-Zanca examined *trk* expression in mouse embryos, what they found was as intriguing as the gene's structure: *trk* was active in a select subset of neurons that grew from embryonic cells called the neural crest. "As far as we could tell," Parada says, "this was the most exquisitely regulated mammalian tyrosine kinase that had been described."

The expression pattern of Trk suggested that it was different from most tyrosine kinase receptors, whose activity causes cells to divide. Indeed, *trk* is active in neurons that will never divide again. This led Parada to wonder if Trk's ligand might be a signal telling neurons to differentiate, not divide.

NGF fills that bill. Despite being called a growth factor, NGF does not promote cell division. Instead it stimulates the sprouting of projections through which neurons contact one another and it supports the long-term survival of nondividing neurons. Moreover, the neurons in which Parada had found Trk are among those that respond to NGF.

Parada began to think Trk might be an NGF receptor. But when he voiced that view more than a year ago, Chao and others shrugged off the idea. For one thing, Trk was too big to fit into the developing NGF-receptor story.

Eric Shooter's lab at Stanford had shown in 1985 that cells that respond to NGF seem to have two types of NGF receptors—one a low-molecular-weight protein of about 80 kilodaltons and the other a higher molecular weight protein of about 140 kilodaltons. Subsequent work by Shooter and others suggested that both were necessary for the high-affinity binding of NGF that seemed to be required for the nerve cell's response. Indeed, at the time, it was widely believed that the low-molecular-weight receptor was actually a part of the larger receptor.

In 1986 Chao's and Shooter's

No coincidence. *In this mouse embryo head, the Trk protein lights up neurons that respond to NGF.*

D. Martin-Zanca, M. Barbacid, and L. F. Parada

groups independently cloned the gene for the low-molecular-weight receptor. Then the search began for an "accessory" protein, with a molecular weight of about 60 kilodaltons, that would combine with the smaller receptor to produce the larger form. Tyrosine kinases seemed to be good candidates because they had been shown to participate in the response to NGF. The putative accessory protein proved elusive, however.

To the NGF community, the Trk protein, even though it was a tyrosine kinase, seemed to be out of the running because, weighing in at about 140 kilodaltons, it is too big to be the accessory protein. But Trk's molecular weight exactly matched that of the larger NGF receptor. Instead of the accessory protein, could it be the entire receptor? It became clear that was the case last fall, when Parada joined forces with David Kaplan, who had just arrived at Frederick. Kaplan had been studying an unidentified 140 kilodalton kinase that was phosphorylated in response to NGF. He and Parada did an experiment that showed his kinase was Trk: They added NGF to a cell line that responds to the factor, and found that the cells' Trk protein was very quickly phosphorylated on tyrosine. That rapid "autophosphorylation" was a telltale sign of the direct activation of Trk by NGF.

With that observation, "David and I were convinced" that Trk was an NGF receptor, Parada recalls. "We wrote a letter to *Nature*, and then I called up Moses Chao, and said, 'Would you like to do an experiment that might change your way of thinking?' "

Indeed, the autophosphorylation finding (*Nature*, 14 March 1991) convinced Chao to enter the fray. In mid-December, with *trk*-expressing cells from Parada and Kaplan, Chao and Hempstead made a key observation. When they exposed the cells to NGF, it became so closely associated with the Trk protein that they could be joined by a chemical linking procedure. "When we saw the results, our jaws just fell," Chao recalls. "Trk behaved as a receptor, and bound NGF by itself.... We had been chasing this high-molecular-weight [receptor] for the last couple of years, without knowing it was Trk."

Meanwhile, Barbacid, who had moved to Squibb, was also searching for the ligand for Trk. Barbacid made the Trk-NGF connection independently, he says, when he saw a paper from Shooter's lab in the January issue of *Neuron* that showed that NGF causes the high-molecular-weight receptor to become phosphorylated on tyrosine. Knowing that Trk is expressed in NGF-responsive cells, Barbacid and his co-workers did their own linking experiments and found Trk bound to NGF. Those results were in the 5 April *Cell*.

While the Parada-Chao and Barbacid groups agree that Trk binds NGF, they disagree about how tightly the two molecules bind, which is a key issue in the NGF field. It has been known for years that NGF binds the low-molecular-weight receptor with a rather low affinity, and that a much tighter binding was needed for NGF to exert its effects.

According to Barbacid, Trk binds NGF tightly enough that he thinks that Trk by itself may give high-affinity binding, something that could suggest Trk alone may carry out the effects of NGF.

And in one simple system it does. In another paper in this issue (p. 558), Eugenio Santos and his colleagues at the National Institutes of Health report that frog eggs, which don't normally respond to NGF, will do so if they have been genetically engineered to express Trk. But Shooter and others warn that such a finding does not mean Trk alone can trigger the specific biochemical pathways required for neuron survival.

In contrast to Barbacid's results, the Parada-Chao team finds that NGF binds to Trk no more tightly than it does to the low-molecular-weight receptor. So how does one get high-affinity binding? They propose that both proteins are necessary—a view supported by a paper they have in this week's *Nature* showing that NGF binds tightly only to cells having both receptors.

That finding appeals to Yves-Alain Barde of the Max Planck Institute in Munich, who has proposed a central role for the low-molecular-weight NGF receptor in mediating responses to what is now known to be a family of nerve survival-promoting factors, collectively called the neurotrophins. In addition to NGF, these include brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3).

Barde proposes that the low-molecular-weight receptor is a common component of the receptors for all the neurotrophins. Last year his lab showed that BDNF binds the low-molecular-weight NGF receptor with the same affinity as does NGF. What's more, the receptor is found in many neurons that don't respond to NGF, including, Barde says, every known neuron-type that responds to BDNF.

If all neurotrophin receptors contain the low-molecular-weight receptor, then where do they get their specificity? For the NGF receptor, the obvious hypothesis is that Trk confers the specificity. And Barbacid's lab has found two additional members of the *trk* gene family, which they call *trkB* and *trkC*. Could the proteins encoded by these genes be the specific components of the BDNF and NT-3 receptors? So far, the researchers are keeping mum about what they have found. But Barbacid dangles a tantalizing hint: "TrkB is the receptor for another factor... but I can't say which." ■ MARCIA BARINAGA

An RNA First: It's

In the old days, every large molecule had its place. Proteins acted as biological catalysts and as building blocks for cell structures; DNA held the blueprints for making proteins; and RNA was just a go-between molecule. But one by one these convenient divisions have disappeared—with RNA's role in life in particular undergoing expansion. And now researchers at the University of Oregon at Eugene and the Instituto di Scienze Biochimiche in Parma, Italy, have expanded RNA's repertoire once again. In work reported on page 542, they show that an RNA molecule can be part of the molecular machinery that transcribes DNA into RNA, an essential step in turning on gene activity.

Until now all of the components isolated from the transcriptional machinery have been proteins. "There are hundreds of examples of transcriptional machines, and not one of them was found to have an RNA," points out Thomas Cech of the University of Colorado at Boulder. Which is why Cech, whose own discovery of catalytic RNA stunned molecular biologists almost a decade ago, winning him a share in the 1989 Nobel Prize, considers the new finding "exceedingly novel. It catches everybody by surprise."

So novel that, at this early stage of the work, no one can really say what the new findings might mean—whether, say, such RNA transcription factors will be widespread in nature or just an oddity. Cech anticipates, however, that the list will soon grow now that researchers know to look for RNA transcription factors.

For Karen Sprague, the leader of the research team that found the RNA transcription factor, the discovery marks the end of a long quest. She first began suspecting that such a factor might exist about 5 years ago when she and her colleagues were analyzing the regulatory regions of the gene for a transfer RNA in the silkworm *Bombyx mori*. The gene's "on" switch, or promoter region, she found, was larger and more complex than had originally been expected. And that made her wonder about the nature of the molecular machine that had to interact with the promoter to begin transcribing the gene.

At the time, the machine was known to contain three proteins: an enzyme called RNA polymerase III that transcribes the DNA into RNA, and two other proteins, simply designated B and C, which connect the transcriptional machinery to the pro-