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-

Part of the Gene-Copying Machinery

moter on the DNA. Were those three proteins enough for the job? Sprague began to doubt it. "The complexity and size of the gene's promoter prepared us for the possibility that the transcriptional machinery was actually larger and more complex than just those three proteins," she says. "It also made us think that the components joined together and acted as one unit."

Sprague knew that some of the other biological machines that operate in converting the information contained in DNA into protein structure are composed of separate parts, including both RNA and protein. In particular, Sprague says, she drew on the work of Joan Steitz at Yale, who had shown that the noncoding material contained in the RNA transcripts of DNA is spliced out by small particles composed of RNA and protein. Sprague also considered ribosomes, the large RNA and protein complexes on which proteins are synthesized, and she had the unorthodox idea that maybe the transcriptional machine for the silkworm gene might also be composed of RNA and proteins. The comparison, she says, was "a leap of the imagination. It wasn't strictly logical."

Oddity or everyman? *How widespread is the RNA transcription factor found in the silkworm?*

As farfetched as the idea seemed at first, her initial results were encouraging. "We actually had evidence for it early on," she says. "But we didn't believe it." Her biochemical analysis indeed gave indications that an RNA molecule might be involved in the gene transcription. In a preliminary study, the group found that the transcriptional machine could be debilitated by treating it with enzymes that digest nucleic acids, specifically RNA. Excited by the findings, the group then tried to isolate the RNA, and that's when everything fell apart.

"The results were messy," says Sprague. They couldn't obtain a pure isolate. "There were two possible explanations," she says. The optimistic view was that the RNA was there, but that they hadn't yet perfected the methods for getting at it. The pessimistic view, she says, was that the original findings were artifactual.

The group abandoned any further plans to go after an RNA component specifically, preferring a more conservative approach instead. "If an RNA was involved, I thought it would just fall out of a straightforward analysis of the transcriptional machinery, and that's what happened," says Sprague. The goal was to take the complex apart, identify all the components, and then put the complex back together again by combining the purified components in the test tube. If this reconstituted complex transcribed the gene, it would be the formal proof that their analysis had succeeded.

But at one point when Sprague and her colleagues attempted to reconstitute the transcriptional machinery, it failed to transcribe the gene. Apparently, in their purification, they had separated away a crucial, but unidentified, transcription factor. The search was on to identify that factor.

Sprague resurrected the old idea that this factor might be an RNA, but she kept the thought to herself. "By this time most of the people on the original project were gone, and I didn't want to bias the results. We looked into the chemical nature of the new factor without any preconceptions," says Sprague. And this time, there was no ambiguity. By all known criteria, the group has identified the missing factor as an RNA molecule.

Not only does the work identify a missing factor, but it fills another "glaring hole," says Steitz. "There are small RNA molecules involved in virtually every area of gene expression," she says. "The only place in this pathway where an RNA-protein particle hadn't been implicated was transcription."

But solving one mystery in this case only opens up more. What could be the role of the newly discovered factor? Sprague notes that the team has not even begun to perform a functional analysis, but there are three avenues she would like to explore. Following on the observations made by Cech, she cites the possibility that the newly discovered RNA transcription factor might itself have a catalytic function. Alternatively, it might provide a structural scaffold on which polymerase III and the other protein components of the transcriptional machinery are hung. As a third possibility, Sprague proposes that the RNA might connect the transcriptional machinery to the DNA template. If so, it might offer a solution to a particular conundrum of transcription by polymerase III.

The precise choreography of the polymerase and its auxiliary factors has posed something of a problem. During transcription, these components are positioned on the template in such a way that the polymerase, as it works its way down the gene, has to barrel through a complex of transcription factors without breaking the complex apart. So a long-standing question has been how these factors remain stuck to the DNA and at the same time allow passage of the polymerase. "One can imagine that the factors would have to move temporarily to allow the polymerase through. In that case, the RNA may act as a tether to keep contact with the template while the other factors move briefly to another site," says Sprague.

Another issue is how RNA has come to be a participant in so many basic cellular functions, including DNA synthesis and protein translocation in addition to protein synthesis and transcription. Steitz proposes that that's because cells are using the materials that became available early in evolution. "When the whole gene expression apparatus got started, all there was, was RNA and protein," she says. "DNA was not really on the scene yet." But there could be another reason as well. "RNA can do many things better than proteins," says Cech. "Its versatility of shape and catalytic abilities approach those of polypeptides. But it also has information content. RNA is such a wonderful, versatile molecule that of course it's going to have a variety of roles."

The task ahead is to find out how widespread these RNA transcription factors are and exactly what they are doing. Whatever researchers find, the discovery of the factors may still provide a lesson about dogma. "Forty or fifty years ago people didn't know which macromolecule was doing what in the cell," says Cech. He cites the example of the *lac* repressor, which inhibits the transcription of genes needed for lactose metabolism in the bacterium *Escherichia coli*. Before it was isolated in 1966, researchers considered the possibility that the repressor might be made of either protein or RNA. But the *lac* repressor and all transcriptional components found until this latest one have been proteins. So, Cech says, people stopped being open-minded to other possibilities. But perhaps it's time to let sleeping dogmatists lie. ■ MICHELLE HOFFMAN