Taking a Direct Path to the Genes

New work suggests that the path by which interferon signals are transmitted from the membrane to the genes in the nucleus is very direct. Could the same be true for other regulatory molecules?

The virus-fighting protein interferon may be turning out to be science's equivalent of some of this summer's Olympic Gold Medalists—performers who had failed to fulfill their brilliant promise at earlier games but now, in their maturity, have suddenly achieved unexpected success. Interferon fits the mold, having first leapt into the public consciousness nearly 15 years ago, buoyed by reports that it might also have cancer-fighting ability. Though that early clinical promise hasn't been fulfilled, interferon research has persisted and is now paying off, albeit in a different way: by offering new—and powerful—insights into how cells work.

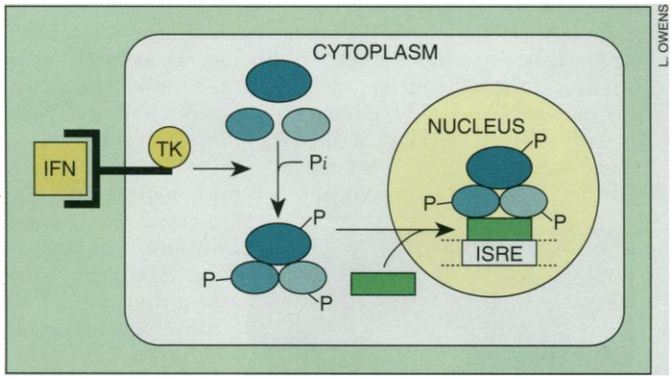
In a series of converging studies, three independent research teams have just traced the pathway by which interferon activates a set of genes thought to be needed for its virus-killing and growth-regulatory actions. And in addition to clarifying the action of interferon, an important part of the body's disease defenses, the work may have a wider significance as well.

Most complete path

Over the past several years, cell biologists have directed a great deal of effort at understanding how the signals conveyed by regulatory molecules docking at their receptors on the cell membrane can be converted into specific changes in gene activity in the nucleus. And while they've made enormous progress, David Levy, a molecular geneticist at New York University School of Medicine, who collaborated on some of the earlier work leading up to the current findings, points out that even the best-studied of these signaling pathways still have gaps. The current results are exciting, says Levy, because the picture they give of the interferon pathway is "the most complete we have so far." What's more,the work may also provide clues to a major mystery in cell signaling. To Levy, the results are "pretty spectacular."

For nearly 40 years now, ever since the Nobel Prize–winning work of the late Earl Sutherland on the hormone epinephrine, it's been a tenet of cell biology that the cellular responses of regulatory molecules, including hormones, neurotransmitters, growth factors, and cytokines such as interferon are mediated by relatively simple cell chemicals such as cyclic AMP and calcium ions. The idea is that when one of these regulatory molecules, carrying messages from outside the cell, attaches to its receptor on the membrane, the receptor's activation leads to an increased concentration inside the cell of cyclic AMP, calcium, or whatever the internal mediator is. Because these chemicals take over the task of conveying the messages in the cell interior, they were dubbed "second messengers."

This picture has been very durable, but as researchers began tracing the signaling pathways for more and more regulatory molecules, they encountered a conundrum. Cell biologist James Darnell of Rockefeller University, the leader of one of the teams doing the current work, explains that while there are "jillions" of molecules sending signals into the cell interior—and evoking their own unique set of responses—only a half-dozen or so second messengers have been identified.



Going for the nucleus. The tyrosine kinase (TK) associated with the interferon-α receptor activates the transcription factor that turns on the responsive genes.

So researchers have been hard-pressed to explain how so many specific signals received at the cell membrane can be converted into specific gene responses in the nucleus with only a few, common second messengers.

But now the answer is in for interferon—and this molecule at least can dispense with a second messenger. What the three teams of researchers have just found is that when interferon contacts its receptor on the cell surface, it immediately activates a protein (known as a transcription factor) inside the cell that then moves quickly into the nucleus where it turns on its own particular set of genes. Says Darnell, "We have a direct link between the receptor and the nucleus." And that, he suggests, provides a much more understandable means of producing specific responses than the use of a few second common messengers.

Indeed, Darnell predicts many other regulatory molecules may also work through similarly direct signaling pathways. Whether he's right remains to be seen, but there's already evidence that interferon-α, the particular type of interferon used for the current experiments, isn't the only cell regulator that operates this way.

Darnell had in fact suspected since the mid-1980s that the interferon-α signaling pathway might not include a second messenger. Work done then by several groups, including Darnell's and that of Ian Kerr and George Stark at the Imperial Cancer Research Fund laboratory in London, had shown that when the interferon binds to its receptor, several genes are rapidly turned on. But, Darnell says, "we were unable to affect the genes that interferon induces with any of the second messenger mimics."

Nevertheless, analysis of the genes that respond to interferon provided a starting point so that the researchers could work backward along the signaling pathway, tracing the steps leading to the gene activation, with the ultimate goal of reaching the interferon receptor. The analysis showed, for example, that all the genes carry a regulatory sequence called the ISRE (interferon-stimulated response element) without which they can't be turned on by interferon's binding to its receptor.

Then, in experiments done in the late 1980s, the Darnell and Kerr-Stark groups were able to identify the transcription factor that causes the gene activation by looking for proteins that bind to the ISRE. Subsequent characterization of the factor by Darnell and his colleagues showed that it is a complex of four different protein subunits, three large ones plus a smaller one, which is the subunit that makes contact with the ISRE.

In cells that haven't been stimulated by interferon-α, the three larger proteins are not linked and are located exclusively in the cytoplasm. But once interferon-α binds to its receptor, they rapidly come together and move into the nucleus where they join up with the smaller protein to make the active transcription factor. The rapidity of the transcription factor activation and gene response

was another hint, Darnell says, that led him to believe that the interferon-α signaling pathway might be very direct. But proving that required knowing more about how the transcription factor was activated in response to interferon-α. The hope was that this would provide a connection between the factor and the receptor on the membrane—and that's where the new work comes in.

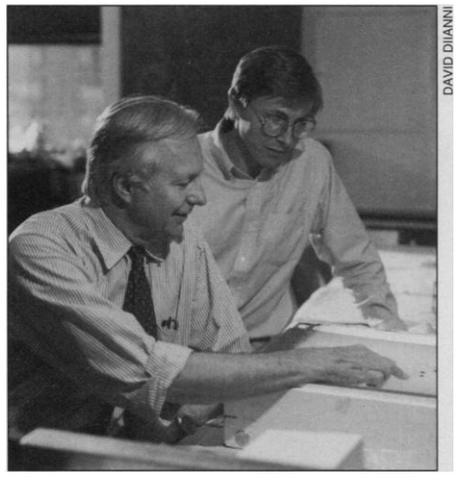
The key to that work was the cloning and sequencing of the genes for the three large transcription factor proteins, work done by Darnell's postdocs Chris Schindler and Xin-Yuan Fu and their colleagues. The sequences revealed that the proteins are clearly related to each other. But when the researchers compared the sequences with those already in the databanks, they didn't find any matches, indicating that the proteins are the first members of a new protein family.

The sequences also provided a clue to the proteins' function. All three of the larger proteins contain an "SH2" domain, a sequence commonly found in proteins that interact with the tyrosine kinases, an important set of enzymes that regulate the activities of other cell proteins by attaching phosphates to the amino acid tyrosine in the target proteins. As Fu, who's now left Darnell's group to set up his own lab at Mount Sinai School of Medicine in New York City, pointed out at the Eighth Annual Meeting on Oncogenes, which was held at the end of June in Fredrick, Maryland: "When you have an SH2 domain, the first thing you ask is whether there is a tyrosine kinase involved."

The fact that the researchers had cloned the genes for the transcription factor proteins also made possible the experiments needed to answer that question. The reason: The cloned genes could be used to make the proteins, which were in turn used to make antibodies for detecting the transcription factor proteins and then following what happens to them in cells treated with interferon.

The upshot of all this is that on page 809, Schindler, Darnell, and their colleagues report that treatment of cells with interferon-α results in a rapid addition of phosphate—to tyrosine residues—in the three large transcription factor proteins. As a result, they associate with one another, enter the nucleus, and then combine with the smaller protein that confers the ability to bind to the ISRE. The Rockefeller researchers have done further work that indicates that phosphorylation is indeed needed to activate the transcription factor. When they blocked the phosphorylation with an inhibitor, the nuclear genes could no longer be turned on in response to interferon-α.

But showing the importance of the tyrosine phosphorylations is only part of the story. There's also the question of what kinase actually carries out the reaction. For while several receptors, especially for growth



DAVID DIIANNI

Following the interferon path. Rockefeller's James Darnell (seated) and Chris Schindler.

factors, have their own built-in tyrosine kinases, the interferon-α receptor doesn't. Enter Sandra Pelligrini and her colleagues at the Pasteur Institute in Paris who, along with Stark, have provided the missing enzyme. (They report their results in the 24 July issue of *Cell*, which also has a paper from Fu.)

Tyrosine kinase found

While a postdoc in the Kerr-Stark lab in London, Pelligrini helped develop a series of mutant cell lines that are unable to respond to interferon-α. In the current work, she's cloned the gene that repairs the biochemical defect underlying the lack of responsiveness in one of the cell lines. Analysis of the sequence of that gene shows that it encodes none other than a tyrosine kinase, designated tyk2, which was originally identified in 1990 by Ricardo Dalla-Favera's group at Columbia University's College of Physicians and Surgeons, but whose function was unknown—until now. The genetic analysis indicates that the enzyme is likely to be the one that carries out the phosphorylation initiated when interferon binds to its receptor, says Stark, who last month moved to the Cleveland Clinic.

Tony Hunter of the Salk Institute in La Jolla, who's an expert on tyrosine kinases, calls the discovery that tyrosine phosphorylation activates a transcription factor "extremely exciting" and "unexpected." There are no other examples, he explains, of transcription factors regulated by tyrosine phosphorylation, although some are regulated by phosphorylation on the amino acids serine or threonine. "We've always assumed that tyrosine phosphorylation was not involved in the ultimate regulatory step [at the gene transcription level]," Hunter says.

Pelligrini and Stark's results don't prove, however, that the tyrosine kinase is in contact with the receptor, although another finding with the mutant lacking the tyrosine kinase certainly points in that direction, Stark says. The mutant also loses its high-affinity binding sites for interferon-α, and that's "a strong clue there is an association between the receptor and the kinase."

What's more, Michael David and Andrew Larner of the Food and Drug Administration's Center for Biologics Evaluation and Research in Rockville, Maryland, have shown that the activation of the transcription factor takes place in the right location (also see page 813). These researchers have for the first time devised a cell-free system that can be used to study interferon action. Such a system has several advantages, says Larner: Researchers can, for example, fractionate cells into their various components—cell membranes, cytoplasm, and nuclei, for example—and see which parts are needed for any particular reaction. And for activation of the transcription factor by interferon-α, the only thing needed is the cell membrane fraction.

"The stories are coming together very nicely and there's no problem in integrating all the data in a consistent model," says Stark, referring to the intersection between his group's work and that of the others. This model shows that as soon as interferon binds its receptor, the tyrosine kinase phosphorylates the three large transcription factor proteins, causing them to link up first with each other. Then in the nucleus they join with the smaller protein, forming the complete transcription factor that turns on the interferon-responsive genes.

The next big question concerns just how widespread this kind of direct signaling pathway is, although evidence is already building that at least one additional receptor works this way. That's the receptor for interferon-γ, which causes activation of a different set of genes having a different regulatory sequence.

Nevertheless, as Schindler, Darnell, and their colleagues report in their *Science* paper, when interferon-γ binds to its receptor, it, too, stimulates a tyrosine phosphorylation, in this case of the 91,000-molecular-weight protein used in the interferon-α transcription factor, but not the other two. The assumption is that the phosphorylated protein serves as a transcription factor for the genes induced by interferon-γ, but Darnell declined to talk about the experiments his lab has done to test this hypothesis since the results have not yet been published.

And there's more evidence as well. Not only has the Darnell group detected genes for additional members of the transcription factor protein family, but there are at least two more kinases (known as JAC1 and JAC2) related to the one that Pelligrini and Stark identified in the interferon-α signaling pathway. Could they play a similar role for other receptors, such as the one for interferon-γ? Such tantalizing questions should prompt enough research to keep scores of postdocs busy for many years to come.

–Jean Marx