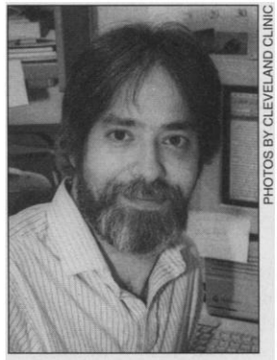
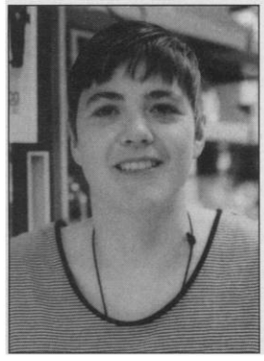
Forging a Path to the Nucleus

In a fast-breaking series of developments, cell biologists have traced out most—maybe all—of the major steps in the Ras pathway for transmitting growth signals to the cell nucleus

The distance from the cell's outer membrane to its nucleus may seem tiny—a matter of only 20 micrometers or so. Yet that minute distance encompasses a major mystery: How do the cells of higher organisms respond to the many growth signals they receive from the environment? The answer is crucial to understanding such longstanding questions in cell biology as what causes cells to begin—and stop—dividing and how may disturbances in those growth regulatory mechanisms give rise to cancer. But much as researchers would love to be able to answer those questions, until recently they have been stumped by a patch of *terra incognita* lying between the specific receptors on the cell membrane, where growth signals dock, and the genes in the nucleus that are turned on in response to the signals at the membrane. They could discern some of the landmarks along the growth signalling pathways, but even the best maps of those pathways contained big gaps.





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Pathway partners. Alan Wolfman and Shonna Moodie.

A remarkable outpouring of reports, primarily in the journals *Cell*, *Nature*, and *Science* during the past 2 months, has, however, filled in the early part of the map of a major growth signaling pathway. These reports have charted the region lying just under the cell membrane, where the Ras protein, a key relay molecule, is activated. Now, on page 1658, a team from the Cleveland Clinic Foundation provides what appears to be the first in another surge of reports that may extend this map all the way to the nucleus. Researchers are being cautious, emphasizing that there are still uncertainties about whether every component of this crucial growth regulatory pathway has been found. Yet even normally cautious members of the cell biology community acknowledge that they are very close to attaining their long-sought goal.

"For the first time we can trace a pathway from the membrane to the nucleus without any obvious gaps," says cell biologist Tony Pawson of the University of Toronto, whose lab is one of several doing the work. Indeed, agrees another of the researchers, Robert Weinberg of the Whitehead Institute in Cambridge, Massachusetts: "It's an extraordinary coalescing of previously disconnected pieces of information. We can now begin to draw the wiring diagram of the pathway [that leads to cell division], connecting its different components."

And while the immediate excitement surrounding these discoveries reflects the intellectual achievement of understanding one of the cell's fundamental growth regulatory pathways, the information may also have a practical payoff. The Ras protein, which is central to the operation of the pathway in question, is encoded by an oncogene that is abnormally activated in several human cancers, including colon cancer. Understanding the full Ras pathway may therefore enable drug designers to develop novel cancer drugs that work by blocking the excessive growth stimulation that might be caused by Ras or other pathway components (*Science*, 14 May, p. 918).

Even though the developments in understanding the Ras growth control pathway are now coming very rapidly, this "overnight sensation," is the culmination of years of hard work dating back to the early 1980s. At the time, researchers had identified several oncogenes on the basis of their ability to make cells cancerous when the genes are mutated or abnormally activated, but they had no idea what the proteins encoded by those genes do normally in the cell. The picture changed dramatically, however, when researchers discovered that certain growth factors and their receptors were the products of known oncogenes. The *erbB* oncogene, for example, turned out to encode the receptor for epidermal growth factor (EGF), and the *sis* oncogene makes a protein chain found in platelet-derived growth factor (PDGF).

Equally intriguing, many of the oncogenes that don't encode growth factors or growth-factor receptors were found to encode proteins that come into play later in the same growth control pathways. Prominent among them was the Ras protein, which serves as a common relay point within the cell for the signals initiated by EGF, PDGF, and a wide variety of other growth factors. But while researchers could work out the rough order in which Ras and the other pathway components come into play, they knew that they were missing several key connections that prevented them from following growth signal transmission all the way to the cell's interior. One key gap came right at the start of the pathway where the growth-factor receptor signals are transmitted to the Ras protein itself.

Once those signals are transmitted, they have a rapid, and very specific, effect on Ras. In the cell, Ras cycles between an active form in which it binds guanosine triphosphate (GTP) and an inactive form in which the third phosphate has been lost from the GTP, converting it to GDP (guanosine diphosphate). Stimulation of the growth factor receptors quickly causes an increase in the cellular Ras-GTP content, indicating that Ras was being activated by the growth factors. The problem was that researchers could see no direct interaction between the receptors and the Ras protein that would account for the change.

It's precisely that void that the first wave of recent work helps to fill in (*Science*, 7 May, p. 767 and 28 May, p. 1338). Researchers from several labs, working sometimes independently and sometimes in collaboration, have shown that in mammalian cells the activated EGF receptor transfers its signals to Ras with the aid of two intermediary proteins, called GRB2 (for growth factor receptor-binding protein) and mammalian son of sevenless (mSOS).* This protein was so named because it is encoded by the mammalian counterpart of the fruit fly "*son of*

*The researchers whose labs contributed to this discovery include: Dafna Bar-Sagi at Cold Spring Harbor Laboratory; David Bowtell of the University of Melbourne in Parkville, Australia; Julian Downward of the Imperial Cancer Research Fund Laboratory in London; Toronto's Pawson; Gerry Rubin of the Howard Hughes Medical Institute at the University of California, Berkeley; Joseph Schlessinger of New York University Medical Center; Robert Weinberg of the Whitehead Institute; and Michael Wigler, also at Cold Spring Harbor.

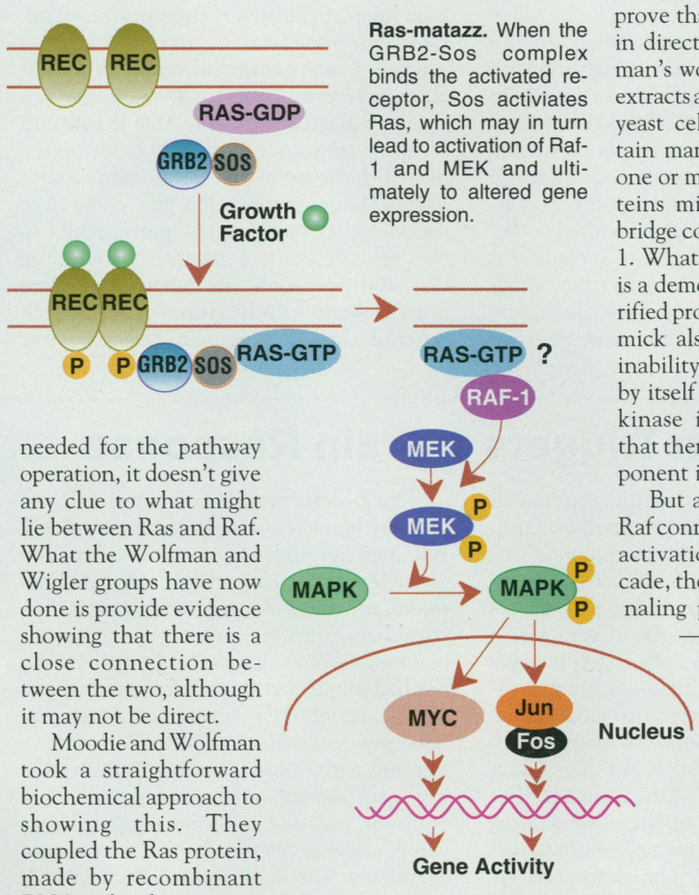
sevenless" gene, which was given its name because it acts after the *sevenless* gene in a pathway regulating fruit fly eye development. Yeast also has its version of the *Sos* gene, known as the *cdc25* gene. Indeed, the genetic studies possible with these simpler organisms laid the groundwork for the mammalian work (*Science*, 27 March 1992, p. 1641).

In a nutshell, the picture that has developed from that work shows that in unstimulated cells, the GRB2 and mSos proteins are linked together in the cytoplasm. The EGF receptor, like many other growth factor receptors, is a tyrosine kinase, an enzyme that adds a phosphate group to the amino acid tyrosine of proteins. And when the receptor is stimulated by a growth factor binding to it, its enzymatic activity is turned on and it phosphorylates itself. Phosphorylation in turn enables the internal segment of the receptor to bind to GRB2 in the GRB2-mSos complex. The Sos protein, which is still attached to GRB2, then binds to Ras, which is anchored to the inside of the cell membrane. Sos activates Ras by causing it to exchange GDP for GTP.

Those results, from a myriad of labs, fill in a long-sought piece of the growth signaling puzzle. But these events, taking place just under the cell membrane, are still a long way from the nucleus. Already building, however, is a new wave of results that may extend the Ras pathway to its ultimate destination. The first indications of this come from Shonna Moodie, Alan Wolfman, and their colleagues at the Cleveland Clinic Foundation, who describe their results in this issue of *Science*, and from Michael Wigler's group at Cold Spring Harbor Laboratory on Long Island, whose paper will appear in the 1 July issue of the *Proceedings of the National Academy of Sciences*. These researchers have provided evidence for a link between activated Ras and a group of cytoplasmic enzymes that have been dubbed mitogen-activated protein (MAP) kinases because they are turned on when cells are stimulated by mitogens, the scientific name for growth factors.

To perform their job, the MAP kinases work together in an orderly sequence. They form a "kinase cascade," a series of enzymes in which each phosphorylates—and thereby activates—the next member of the series. But the big question was, How do mitogen signals get transmitted to this kinase cascade? Last fall, two groups, one led by Thomas Sturgill of the University of Virginia in Charlottesville and the other by Joseph Avruch of Harvard's Massachusetts General Hospital, provided one clue by showing that the product of another oncogene, *raf-1*, feeds into the pathway. They found that the Raf-1 protein, which is itself a protein kinase, phosphorylates and activates an enzyme, called either MAP kinase or MEK, located near the start of the cascade.

That information helped push the start of the cascade back closer to the membrane, especially since there was already reason to believe Raf might come into the growth regulatory pathway after Ras. Part of the evidence for that idea was that mutations that prevent Raf from functioning also prevent the cancerous transformation of cells by Ras. But while that evidence shows that active Raf is needed for the pathway operation, it doesn't give any clue to what might lie between Ras and Raf. What the Wolfman and Wigler groups have now done is provide evidence showing that there is a close connection between the two, although it may not be direct.



Ras-matazz. When the GRB2-Sos complex binds the activated receptor, Sos activiates Ras, which may in turn lead to activation of Raf-1 and MEK and ultimately to altered gene expression.

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Moodie and Wolfman took a straightforward biochemical approach to showing this. They coupled the Ras protein, made by recombinant DNA technology, to tiny silica beads and then incubated the beads with cell extracts. The result: Raf-1 from the extracts binds to Ras, but only when Ras is in its active, GTP-binding state. Also in the complex is the active form of the MEK enzyme, suggesting that Ras binding to Raf may lead to MEK activation. "It's the first demonstration that you can actually see specific signaling complexes of Ras and Raf," says Wolfman. Wigler and his colleagues came to a similar conclusion by a different route, using the "two-hybrid" system in yeast, which detects interacting proteins by virtue of their ability to turn on a gene that will produce an intense blue dye.

While Wolfman and Wigler may be the first to get their results into print, they definitely aren't the only researchers now finding a Ras-Raf interaction. *Science* has learned that at least four other groups, including Avruch's at Massachusetts General, Julian Downward's at the Imperial Cancer Research Fund Laboratory in London, Jonathan Cooper's at the Fred Hutchinson Cancer Research Center in Seattle, and Frank McCormick's at Onyx Pharmaceuticals in Richmond, California, have either submitted papers to journals or will soon.

Still, Wolfman and Wigler sound a note of caution. They point out that while the results so far show a close connection between Ras and Raf-1, they don't prove that the two proteins are in direct contact. Since Wolfman's work was done with cell extracts and Wigler's with whole yeast cells, their systems contain many other proteins, and one or more of these other proteins might have served as a bridge connecting Ras and Raf-1. What's needed, Wigler says, is a demonstration that the purified proteins interact. McCormick also points out that the inability so far to show that Ras by itself can activate the Raf-1 kinase is another indication that there might be a third component in the complex.

But assuming that the Ras-Raf connection does lead to the activation of the kinase cascade, the goal of the growth signaling pathway—the nucleus—is in sight. The last kinase in the cascade phosphorylates several cell proteins that may bring about the cell's responses. Some of these act in the cytoplasm, but others are transcription factors: proteins that help turn on gene expression. The protein encoded by the *myc* oncogene is, for example, a transcription factor, and Roger Davis and his colleagues at the Howard Hughes Medical Institute at the University of Massachusetts Medical School in Worcester have found that MAP kinase phosphorylates Myc, thereby enhancing its ability to stimulate gene activity. Other transcription factors that may be activated by MAP kinase phosphorylation include the products of the *jun* and *ets* oncogenes. These findings all reinforce the notion that the cell's growth pathways are liberally paved with oncogene products, although when it comes to human cancers only abnormal Ras activation seems to play a major role.

In any event, the outline of the Ras signaling pathway from the membrane to the nucleus seems clear: EGF binds to its receptor; the receptor then binds the GRB2-Sos complex, which in turn activates Ras; Ras activates the Raf-1 kinase, with or without

help from a small number of other proteins; Raf-1 activates the rest of the MAP kinase cascade, finally culminating in the phosphorylation of transcription factors and other cellular proteins that bring about the cell's responses. As Weinberg puts it, "It's a simple linear progression. It has many steps, but many of those steps are occupied by well-known friends"—among them the oncogene products that he and other researchers have been studying for years.

Still, while the general outline of the pathway may be relatively straightforward, there are sure to be complications. As Wigler says, the path is not just a "bucket brigade," in which one component simply passes the signal to the next. For one thing, there are already indications of branch points and alternate routes. One such example comes from Moodie and Wolfman, who could still get activation of MEK in cells from which they had depleted the Raf protein, indicating that cells contain another enzyme that can do the job. The most likely candidate: an enzyme called MEK kinase, recently described by Gary Johnson of the National Jewish Center for Immunology and Respiratory Medicine in Denver and his colleagues (*Science*, 16 April, p. 315).

What's more, the GRB2-Sos complex does not always have to interact directly with a tyrosine kinase growth factor receptor, as it does in the case of the EGF receptor. Papers from Joseph Schlessinger's group at New York University Medical Center and that of Michael Czech of the University of Massachusetts Medical Center in Worcester, which are in press in *Science*, show that the insulin receptor transmits its signals to the complex with the aid of two additional proteins. Still another complication may come from the fact that many of the participants in the growth control pathway, including Ras, Raf and MEK, are members of large protein families. "Which ones really see each other [in the cell] is going to be hard to sort out," says Wigler. Downward agrees that the pathway will have its complications. "It would be a mistake to think we know how it all works," he says. Still, the way the work is going makes him optimistic that it can be done. "We have a good model with the EGF receptor, and that's a good place to start," he concludes.

It's still too early to tell whether the information will pay off in a practical way with new cancer drugs. Meanwhile, Wigler says, the discoveries point to a fundamental cell biology lesson. The pathway was very highly conserved during evolution, he notes, since it's found in species as diverse as yeast, the fruit fly, and the roundworm *Caenorhabditis elegans*. The Ras pathway and kinase cascade weren't just "jury-rigged" during the course of mammalian evolution, Wigler points out, "but were a stable solution to a problem"—how to relay signals, whether for growth or development, to the cell interior.

–Jean Marx

NEUROSCIENCE

Magnetism Triggers a Brain Response

From before birth until death, the human brain is bathed in magnetic fields. Earth itself generates a pervasive field, and human technology adds electromagnetic fields from devices ranging from high-tension power lines to hair dryers. Does the brain respond to these subtle fields? The notion that humans can perceive magnetic fields, as homing pigeons seem to do, has remained more than a little controversial (*Science*, 15 May 1992, p. 967). Also controversial is the possibility, posed by a few epidemiological studies, that electromagnetic fields might cause cancer, in the brain and other tissues. But now a pair of geophysicists, taking a break from studying magnetism in rocks, may have cast some light into this confusion.

Working with neurosurgeon Hans Wieser at University Hospital in Zurich, Michael Fuller of the University of California, Santa Barbara, and Jon Paul Dobson of the Swiss Federal Institute of Technology have measured what seems to be a distinct physiological response in human brains exposed to weak magnetic fields. The study says nothing about the alleged dangers of such fields, and it has its share of caveats—from a small sample size to the not entirely normal brains involved. Furthermore, the study hasn't been vetted by the full community of researchers who study the subject, since details have not spread beyond last month's meeting of the American Geophysical Union. Still, some researchers in human magnetoreception are intrigued. Says Andrew Marino of the Louisiana State University Medical Center in Shreveport: "It [sounds like] a very interesting observation and merits following up."

The Zurich researchers aren't the first to look for brain responses to magnetic fields. But in earlier studies, researchers listened in on subjects' brain activity through scalp electrodes, and the results were inconclusive. During a chance meeting, however, Fuller learned of a more promising possibility. Fuller, who has had a long and prominent career studying the record of Earth's changing magnetic field, was on a sabbatical at the Zurich institute and, with Dobson, was interested in looking into human magnetoreception. Over coffee an institute colleague suggested they contact Wieser, a specialist in the treatment of epilepsy. Wieser, the colleague said, would have just the technical setup they needed.

What Wieser had were patients with electrodes inserted into their brains as a first step in treating epileptic symptoms that had not responded to drug treatment. The only recourse for these patients is surgical removal of small portions of the brain (usually in the hippocampus) that generate the storms of brain activity responsible for their seizures. The electrodes are crucial to precisely locating the regions requiring excision. Coincidentally, Fuller and Dobson realized, they could serve as particularly sensitive monitors of any physiological activity elicited by a magnetic field.

Working with Wieser, Fuller and Dobson enclosed each patient's head in a pair of direct-current coils that generated a magnetic field of 1 to 2 milliteslas. Such fields are 100 times stronger than Earth's, approaching the strength of fields induced by household appliances, says Fuller. To their surprise, all three patients tested so far showed an apparent response to the induced magnetic field.

The first patient had a total of 25 bursts of epileptiform—epilepsy-like—activity during the 10-second periods after the field came on, compared with only three during the preceding 10-second periods. The second patient's counts were none before and 15 after. The third patient had had no bursts of activity during 2 full days of monitoring, but activity began within seconds of applying the field.

These preliminary results "do seem to indicate that we have induced some epileptiform activity," says Dobson. However, "the physiological mechanism is something we don't understand yet." The researchers speculate that the magnetic field could be triggering activity directly, by changing the flow of ions through nerve cell membranes, or indirectly, perhaps by way of the microscopic bits of the mineral magnetite recently found in human brains (*Science*, 15 May 1992, p. 967). Both mechanisms, however, fail to explain the roughly 5-second delay seen between the onset of the field and the first bursts of activity.

Still, says Fuller, "I don't think this is an artifact." He adds that "even if it is, it's a beneficial one." Neurosurgeons now have to monitor epilepsy patients for days or even weeks, waiting for the problem area in the brain to reveal itself, but by triggering activity with a magnetic field, doctors might be able to track down the key area in a few minutes. And if the effect does hold up, it could offer insights into how our brains, like those of navigating birds, might "feel" the unseen magnetic fields that surround us from birth to death.

–Richard A. Kerr