Two Major Signal Pathways Linked

Researchers get a handle on the biochemical connection between the second messenger cyclic AMP and the Ras pathway for transmitting growth signals

Over the past year, cell biologists achieved a signal success. In a remarkable outpouring of papers, they traced the molecular chain that constitutes one of the major "signal transduction" pathways by which the messages carried by growth factors are sent from the cell membrane to the cell interior, where the messages are acted on. The chain in question is known as "Ras pathway," because the protein product of the *ras* oncogene is one of its key links (*Science*, 11 June, p. 1588). The achievement was hailed as a major step not only because of what it revealed about normal cell growth control, but also because of the possibility that the information could be used to devise better therapies for the cancers in which the Ras pathway is abnormally activated. Significant as the achievement is, however, it represents only the first level of understanding how cellular activities are regulated.

There are many more levels yet to be explored because in its natural environment, the cell is awash in a sea of messages from hormones, cytokines, and a host of other molecules in addition to growth factors. All must be transmitted through intermediates to the cell's interior. Since the operation of the cell requires that these multiple signal pathways be coordinated, researchers need to find out how the pathways manage complex biological "crosstalk." Now, in another remarkable surge of reports, researchers from several labs in the United States and Europe have taken a big step toward explaining how the Ras pathway interconnects with another major signaling pathway. (Two of the groups describe their results on pages 1065 and 1069.)

What all these groups have shown is that a compound known as cyclic AMP, which serves as a "second messenger" for transmitting the signals of a diverse group of hormones, can influence transmission of growth signals through the Ras pathway. "It looks like a very interesting type of crosstalk between the two types of pathway," says Edwin Krebs of the University of Washington School of Medicine in Seattle, who is a lifelong pioneer in studying cyclic AMP's action (he shared the 1992 Nobel Prize for his contributions) and whose lab is one of those doing the current work. Pharmacologist Gary Johnson of the National Jewish Center for Immunology in Denver, whose lab is also looking into the connection, says, "You're going to see a lot of this. People are really going to jump on the bandwagon."

Aside from the intrinsic fascination of understanding how the signal transduction pathways communicate, these new findings are intriguing because they may account for a puzzling aspect of cyclic AMP's action. Originally identified in the late 1950s by another Nobelist, the late Earl Sutherland, cyclic AMP stimulates cell growth in some cells, while inhibiting it in others. Until now, the reasons for that apparent paradox have been unclear, but the new results may provide a biochemical basis for it. What's more, the work could lead to better and more specific methods of inhibiting the abnormal cell growth that underlies cancer and atherosclerosis.

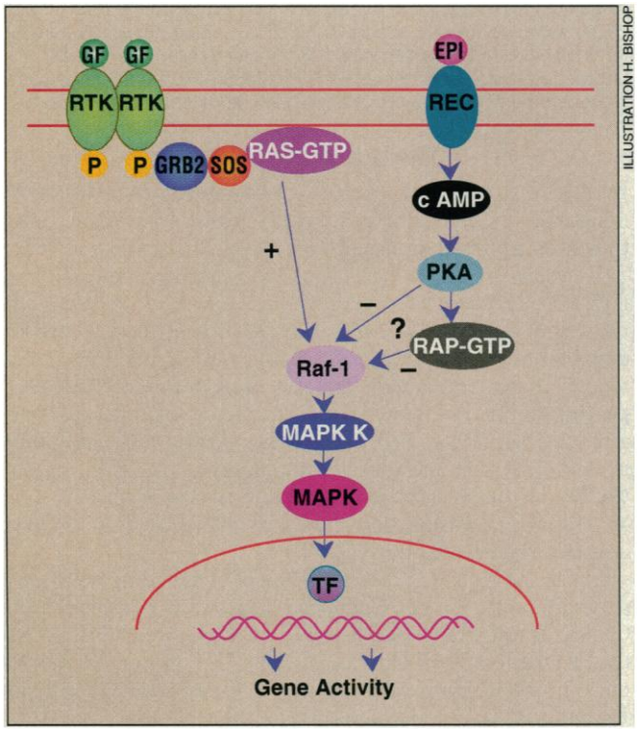


ILLUSTRATION H. BISHOP

Getting together. In some cells, cyclic AMP, possibly acting through protein kinase A (PKA), blocks transmission of signals from Ras to Raf-1 and thereby prevents activation of the MAP kinase cascade.

The five groups who have made it into print so far* have all demonstrated that anything that increases cyclic AMP concentrations in the cells they are studying inhibits transmission of growth stimulatory signals through the Ras pathway. "We demonstrated the connection by choosing straightforward ways to elevate cyclic AMP and seeing how that affected the pathway," says Thomas Sturgill of the University of Virginia Health Sciences Center in Charlottesville. Those straightforward ways include treating the cells with hormones that increase cyclic AMP concentrations or with inhibitors that prevent its breakdown.

The five groups have also been able to determine approximately where cyclic AMP blocks the Ras pathway. The earlier work tracing that pathway revealed that signals are transmitted from Ras to the protein product of another oncogene called *raf-1* and from there to a series of enzymes called the mitogen activated protein (MAP) kinases. When the MAP kinases are activated in response to growth factors, they control the activity of other enzymes by attaching phosphate groups to their targets. It's through this activity that the MAP kinases bring about many of the cellular responses to growth factors.

Knowing that cyclic AMP inhibits the MAP kinases was an important step, but it wasn't by any means specific enough—since there are many steps in the pathway between the receptors where growth factors first exert their effects and the first MAP kinase. To pin down the location of the blockage more precisely, the researchers looked at individual steps in the pathway to see which one was affected. The consensus conclusion from the five groups: The block-

*Krebs' team, including Lee Graves, Karin Bornfeldt, and Russell Ross, all at the University of Washington School of Medicine, published in the 1 November *Proceedings of the National Academy of Sciences*, as did a team led by John Lawrence of Washington University School of Medicine in St. Louis; a group led by Johannes Bos of Utrecht University in the Netherlands has a paper in the November *EMBO Journal*; Sturgill's group and that of Simon Cook and Frank McCormick at Onyx Pharmaceuticals in Richmond, California, have the *Science* papers.

age lies somewhere between Ras and Raf-1. "Ras is basically backed up, sitting there in the active state with nowhere to go," says Onyx Pharmaceuticals' Frank McCormick.

Although researchers have yet to pin down the mechanism of this blockage, the fact that it occurs, inhibiting the Ras pathway, is consistent with cyclic AMP's known ability to depress the growth of the cell types that these five groups used: certain strains of fibroblasts (found in connective tissue), fat cells, and smooth muscle cells. But while this first wave of papers consistently shows an inhibitory effect of cyclic AMP on the Ras pathway, cyclic AMP's growth stimulation in other cell types raises the question of whether it might increase the Ras pathway's activity in other circumstances. The answer appears to be yes.

At least two groups (who have not yet gotten their papers into the journals), have results indicating that in the neuron-like PC12 cells, elevated cyclic AMP concentrations stimulate MAP kinase activity. One group includes Emmanuel van Obberghen, Morten Frödin, and Pascal Peraldi of the INSERM lab at the Medical School in Nice, France, who presented their data at the FASEB meeting in July and who have a paper in press at the *Journal of Biological Chemistry*. The other work comes from Jeremy Tavaré's team at the University of Bristol in England. Although their results might seem to contradict the others, they don't. In fact, says van Obberghen, "the nice thing is that the biology makes perfect sense."

In contrast to the cells studied by the initial five groups, in which cyclic AMP antagonizes growth factor action, in PC12 cells it has the same effect as nerve growth factor—stimulating neuronal differentiation. And since nerve growth factor works through the MAP kinases, it's logical to think cyclic AMP would also do so, although the explanation for cyclic AMP's different effect in PC12 cells is still unclear. As McCormick points out, however, the new results should help researchers solve the mystery by telling them where to look for the biochemical events that account for the difference.

The rapidly accumulating results leave no doubt that crosstalk is taking place between cyclic AMP and the Ras pathway. Nevertheless, everyone agrees that the full significance of the findings remains to be determined. Signaling pathway expert Philip Cohen of the University of Dundee, Scotland, calls the results "quite interesting," but he adds that "we don't know what the mechanism is, or what it means physiologically. It's very early days indeed."

As Cohen's remarks suggest, one very desirable additional piece of information would be the mechanism by which cyclic AMP affects the Ras pathway. The Sturgill group's work points to one possibility—at least for the inhibitory effects. Cyclic AMP exerts many of its effects in the cell by activating another regulatory kinase, protein kinase A (PKA). Sturgill and his colleagues have evidence suggesting that PKA phosphorylation of Raf-1 may be what causes the blockage in the Ras pathway. Consistent with this suggestion is the finding by John Lawrence's group at Washington University School of Medicine that cyclic AMP's inhibitory effect requires an active PKA.

Beyond answering fundamental questions about pathway crosstalk, the work also has the potential for clinical application. "I think it has very broad significance," says the University of Washington's Graves. "It's the first physiological example of inhibition of the [Ras] pathway." And that's of great potential import because it might be possible to use the information to devise ways of inhibiting the abnormal cell growth of those cancers in which the Ras pathway is overactive by stimulating cyclic AMP production in tumor cells or finding drugs that mimic its effects on the MAP kinases. Atherosclerosis, a major contributor to heart attacks and stroke, might be another target, since the Washington group saw the inhibitory effect in smooth muscle cells, whose abnormal proliferation in the artery walls may lead to the formation of atherosclerotic plaques.

Particularly encouraging from the point of view of drug development is the specificity of cyclic AMP's inhibitory effects. "The important thing," Johnson says, "is that it inhibits one component—uncoupling Ras from Raf—but other upstream functions can remain intact." That may mean that cancer drugs based on mimicking cyclic AMP's action will have fewer side effects than do those currently in use. If so, then Johnson's predicted bandwagon should soon be getting up a pretty good head of steam.

–Jean Marx

ATMOSPHERIC SCIENCE

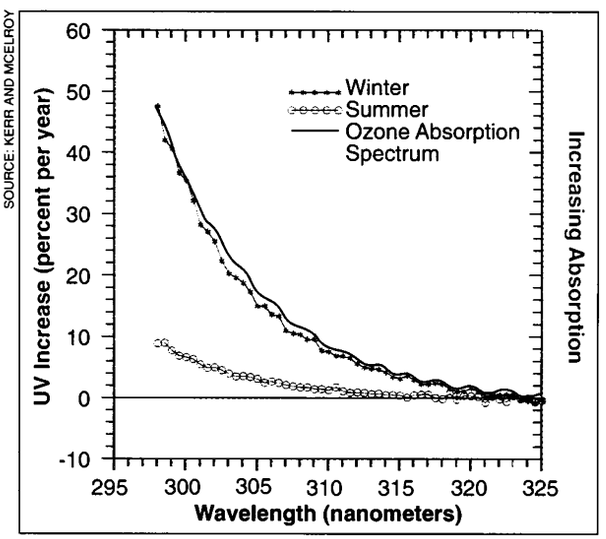
Filling a Hole in the Ozone Argument

Most researchers are expecting an increase in harmful ultraviolet radiation over the coming years, as the protective stratospheric ozone layer thins, eroded by manmade chlorine compounds. That's the reason stock in sunscreen makers looks like such a good bet at the moment. Yet a small, vocal group of dissenters insists this scenario has a hole as big as the "ozone hole" that forms every year over Antarctica: In spite of years of ozone loss, the only site that shows a long-term increase in sunburning rays is Antarctica itself. Indeed, a 1988 study even found a slight *decrease* in harmful ultraviolet rays at eight U.S. sites between 1974 and 1985.

A paper in this issue of *Science* may help close this gap in the ozone argument. On page 1032, James Kerr and Thomas McElroy of Canada's Atmospheric Environment Service show that at a carefully monitored site in Toronto, wintertime levels of ultraviolet-B (UV-B) radiation—the skin-damaging wavelengths that ozone ordinarily soaks up—increased more than 5% every year from 1989 to 1993, as ozone levels dropped. "It's the closing of the loop," says Kerr.

John Frederick of the University of Chicago, who studies ultraviolet light near the Antarctic ozone hole, agrees. "It's important that this result get in the literature," he says, since skeptics have argued that air pollution and clouds, by absorbing ultraviolet light, may negate the effects of ozone loss, making it less urgent to protect the ozone layer. "There's been too much loose talk," he adds. But the result may not end the debate, since critics argue that the Canadian results could stem from unusual atmospheric conditions.

One reason all sides are taking the new results seriously is the care that Kerr and McElroy took to ensure that any UV-B changes recorded over time reflect a real trend, and not simply a drift in the sensitivity of the instrument. In developing the instrument, says Kerr, he and McElroy took "a fair bit of care to stabilize the electronics" that detect the light. They also recalibrated it every month or so by testing its response to a battery of standard lamps.



Telltale match. Ultraviolet light has increased most at the wavelengths most strongly absorbed by ozone *(red)*.