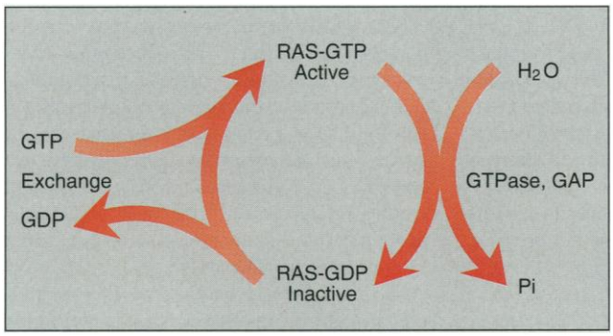
New Genes May Shed Light On Cell Growth Control

One of the most exciting developments in molecular biology in the past several years has been the convergence of two independent lines of work: research on the cancer-causing oncogenes and investigations of the signaling pathways that carry messages telling cells to start—or stop—dividing. Convergence was achieved when cell biologists found that the pathways that transmit those signals into the cell are heavily populated by the proteins made by the several dozen oncogenes currently known. But to understand fully how cell signaling is carried out—information that might aid in designing therapies to halt cancer growth—those same researchers now want more nitty-gritty detail on how oncogene-proteins are turned on and off, and how they bring about their cell responses. Which brings us to the oncogene called *ras*.



Rolling along. An exchange or releasing protein must remove GDP from the Ras protein before Ras can be reactivated.

Of all the proteins molecular biologists are interested in, none is more central than the one produced by *ras*. Not only is the Ras protein a common relay point for signals from all the growth factor receptors looked at so far, but mutations that lock Ras in the permanent "on" position apparently contribute to the development of several types of cancer, including colon cancer. Yet while researchers have made progress in understanding how Ras works (*Science*, 27 March, p. 1640), key pieces of the puzzle have remained missing. Now, though, one of those missing pieces has been identified.

Researchers at several labs around the world have cloned the first mammalian genes for proteins known as the "Ras exchangers"—proteins that may turn Ras on in response to the action of growth factors. "The linkage between growth factor receptor activation and Ras has been really obscure," explains oncogene expert Robert Weinberg of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, adding that "now the discovery of these exchangers may have filled in the missing link."

Frank McCormick of Onyx Pharmaceuticals in Emeryville, California, whose work focuses on Ras, agrees with Weinberg about the finding's importance and points out that the new information "has all sorts of therapeutic possibilities." Drugs that inhibit Ras activation might be used, for example, to treat diseases in which growth stimulatory pathways are excessively active. Though such conditions may include cancer, McCormick cites neurofibromatosis, a hereditary disease in which failure to turn off Ras activity leads to mental retardation as well as to numerous benign tumors, as an even better candidate for Ras-based therapy.

As is often the case with the cloning of mammalian genes, the researchers doing the Ras-exchanger work got a big boost from the fact that the genes for similar proteins had already been discovered in yeast and *Drosophila*. Previous work had shown that Ras, whether in mammalian or yeast or *Drosophila* cells, can transmit its signals only when it has bound to the nucleotide GTP (for guanosine triphosphate).

But continuous transmission of these signals isn't a good thing—witness the mutations that lead to cancer by causing Ras to remain on all the time. That does not normally happen, however, because Ras is inactivated through the intercession of another protein, known as GAP (for GTPase-activating protein), that stimulates GTP breakdown, yielding guanosine diphosphate (GDP), which remains tightly bound to the now inactive Ras, plus inorganic phosphate.

It would be equally bad, however, if Ras could not be activated at all. So to permit Ras to pass back to the active state when a growth signal is to be transmitted, so-called exchange proteins are needed—ones that would release the GDP, allowing its replacement by GTP. "The intrinsic release [of GDP] is so slow you could never have significant exchange without such factors," explains molecular biologist Larry Feig of Tufts University, the leader of one of the groups that have cloned mammalian exchange factors.

As Feig's remarks suggest, it has seemed clear for some time that there must be Ras exchangers in mammalian cells, similar to those already found in yeast and the fruit fly. But while several groups have obtained indirect evidence for mammalian Ras exchange proteins in the past few years, no one had been able to isolate them—and so Feig took his cue from the yeast work.

Examination of exchange proteins obtained from two different yeast species showed that certain parts of the proteins were quite similar, Feig says. He and his colleagues chose a segment that was particularly highly conserved, and assuming that, because of its important function, it would also be highly conserved in mammals, made oligonucleotide probes that could be used to fish out any corresponding clones from a library of cDNAs representing all the genes expressed in rat brain.

With the aid of the yeast-based probes, Feig and his colleagues, Chengchao Shou and Charles Farnsworth, both of Tufts, and Benjamin Neel of Beth Israel Hospital in Boston, eventually cloned a gene for a large protein (it has a molecular weight of 140,000) that they've now shown to have the exchanger activity they were interested in. (The results appear in the 23 July *Nature*.) When they used the cloned gene to make the segment of the rat protein containing the region that is structurally similar to the yeast exchangers, they found that the protein stimulates the release of GDP from Ras, Feig says, but not from two other members of the Ras superfamily that have different functions and presumably their own exchangers.

At the same time Feig's group was looking for the rat Ras exchanger, Enzo Martegani and his colleagues at the University of Milan, Italy, were on the track of the exchanger from a different species, namely the mouse. And while they also used a somewhat different strategy, they ultimately came up with what appears to be the same gene as Feig.

Martegani's group looked for mouse cDNAs that would cure a mutation that knocked out the *CDC25* Ras exchanger gene in the yeast *Saccharomyces cerevisiae*. The original clone they found, which they described in the June issue of the *EMBO Journal*, did not contain the entire gene, although it included enough of the sequence to restore Ras exchanger activity in the yeast. In more recent work that is in press at the *EMBO Journal*, Hui Cen and Douglas Lowy of the National Cancer Institute (NCI), working with Renata Zippel of the Milan group, have cloned at least an almost full-length version of the mouse gene and have confirmed its identity to Feig's. "Larry's gene is a little bigger at the N-terminal, but otherwise I don't think there are any other substantial differences," Lowy says.

Whether in the rat version or its mouse equivalent, the new gene has some intrigu-

ing features. For one thing, Feig says, one segment of the exchange protein encoded by the gene turns out to have an unexpected resemblance to a sequence found in the proteins encoded by two other oncogenes, designated *dbl* and *bcr*. What's interesting about that is that last year, Richard Cerione of Cornell University and his colleagues showed that the *dbl* protein is itself an exchanger for a member of the *rho* subgroup of GTP-binding proteins, which are distantly related to Ras and thought to be involved in assembling the network of protein filaments that give cells their shape. Feig's protein may therefore be a "two-headed exchanger," Cerione says, that can connect Ras and *rho* protein activities. That might help explain, for example, the changes in shape that cells undergo when stimulated by growth factors.

What's more, both the Feig and Lowy groups find that the new Ras exchanger is made only in brain cells. Since Ras proteins themselves are made in all the cells of the body, it would seem that there are additional exchangers for Ras in other tissues, Feig says. And indeed, David Bowtell of the University of Melbourne and his colleagues have just cloned two genes, which are the mouse equivalents of the fruit fly exchanger gene designated *sos*. And these are not only different from the one discovered by the Feig and Lowy groups but, Bowtell says, "They're expressed everywhere we've looked so far." In addition, still another oncogene, called *vav*, encodes a protein product that also has Dbl- and Bcr-like sequences and might therefore be another exchanger for the *rho*-like proteins.

The new findings should give cell biologists a much more complete picture of how Ras is regulated, and perhaps about its interactions with other signaling molecules as well. In fact, the results could have a bearing on the way that people view signaling pathways. "People used to think in terms of linear [signaling] paths, but it's probably going to be a lot more complicated than anyone imagined," says Cerione, referring to the possibility of connections between proteins such as Ras and Rho. But they will have to do a lot of hard work to sort out the precise functions and interactions of all the exchange proteins they are identifying.

One of the immediate goals will be to find out whether growth factor receptors activate the Ras exchanger, as is now generally assumed. "They [the exchangers] certainly activate Ras," McCormick says. "The question is, How are the exchangers activated?" He points out they don't necessarily have to be activated by the receptors since the receptors might also stimulate Ras activity by preventing the stimulation of its GTP breakdown by GAP.

Nevertheless, researchers, including Lowy's group and also that of Tohru Kamata of the NCI-Frederick Cancer Research and Development Center, have circumstantial evidence that growth factors, such as nerve growth factor, work by increasing Ras exchanger activity. The exchanger protein structure itself isn't providing any clues about any potential interactions between the exchangers and growth factor receptors, however. "As far as I can tell from the sequence, there are no direct connections to the receptors," Feig says. That suggests, he says, that another protein will have to relay the receptor signals to the exchanger.

Of course, having the exchanger proteins in hand should make it easier to answer the questions about exchanger functions, since it will now be possible to make specific antibodies that can be used to follow more directly what happens to the exchanger proteins when growth factors interact with their receptors. But whatever the outcome of such studies, from the work already done, it's clear that studies of exchange proteins for Ras and its relatives are moving into a new, high-growth phase.

–Jean Marx

SOLAR PHYSICS

GRO Shows Particles in a Magnetic Trap

When the sun erupts in a solar flare, a surge of gamma rays accompanies the visible brightening and the bursts of charged particles. During the intense solar flares of 1991, those gamma emissions nearly blinded the sensitive eyes of the orbiting Gamma-Ray Observatory (GRO), designed to pick up trickles of gamma photons from the edges of the universe, says University of New Hampshire solar physicist James Ryan. But the versatile Comptel and Egret detectors aboard the orbiting observatory not only withstood the heat of last year's biggest solar flares; they also brought back a major discovery—a "gamma-ray afterglow" that persists after the flare is over. The afterglow, solar physicists say, offers a clue to the still mysterious nature of solar flares.

BOGDAN ROMPOLT

All bottled up. Bright gases in the solar corona trace magnetic loops that confine charged particles.

The GRO instrument observed persistent gamma rays from two flares, both in June of 1991. While the flares appeared to last for just minutes in visible light, the gamma rays kept coming out for 5 hours from one flare and 90 minutes from the other. This afterglow, says principal investigator Ryan, shows that protons accelerated by the poorly understood process of a flare get trapped by magnetic fields in the sun's atmosphere.

The trapping, he thinks, takes place in giant magnetic "bottles" formed by magnetic force lines coiled into a slinky some 15 times the diameter of Earth. After bouncing "zillions of times" through the coils, says Ryan, the particles slowly leak out, colliding with other particles in the solar atmosphere and generating the afterglow.

Scientists already knew that loops in the sun's magnetic field can channel particles accelerated during solar flares. But the long-lasting trapping was a surprise, says Ryan. Theorists had first proposed that magnetic confinement might play a role in flares back in the 1960s, says solar astronomer Carol Jo Crannell of the NASA Goddard Space Flight Center. The original idea was that the trapping causes flares: Somehow the magnetic bottles break open, releasing the particles and setting off a blast of other particles and radiation. "People tried to discredit that by showing the trapping couldn't occur," Crannell says. "Jim [Ryan] showed that the trapping can and does happen," even though the findings don't support the idea that the opening of the magnetic trap causes the flare. If that were the case, GRO would have seen the gamma rays—a sign of the trapping—before each flare, not afterward.

The observations may, however, provide a clue to the origin of at least one component of flares proper. Besides generating a gamma afterglow, the trapping may also cause the gamma-ray emissions during the main part of a flare, says Ryan, as trapped protons slowly leak out the bottom of the loops.

"We are understanding how they [solar flares] behave," says Ryan. But scientists still have no idea what makes these particles suddenly start whirling through the magnetic slinky in the first place, he admits. "Our understanding is surprisingly meager."

–Faye Flam