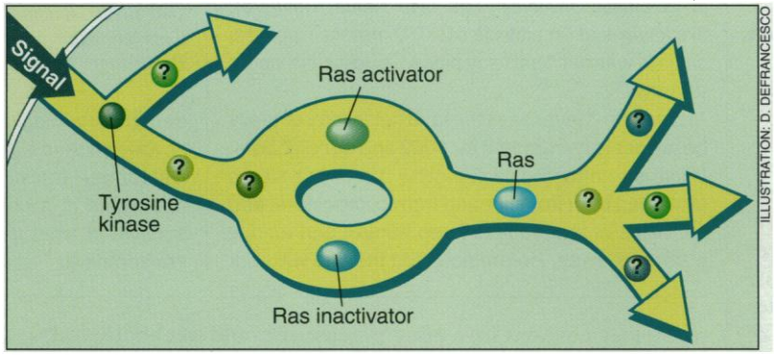
Signals Into Unknown Territory

Oncogene researchers and developmental geneticists used to inhabit separate territories, but work on cellular signal transduction pathways is bringing them closer together

GRADUATE STUDENT SCOTT CLARK AND postdoc Michael Stern thought they were working on entirely different projects in developmental biology—and neither thought his work had much to do with cancer. So it came as a surprise early last year when the pair found out they were wrong on both counts.

Clark and Stern worked literally back to back at separate benches in the Massachusetts Institute of Technology lab of developmental biologist Robert Horvitz. Clark was studying the genetics of sex organ development in the nematode *C. elegans*. Stern, who is now an assistant professor at Yale, was looking for genes required for the worm's muscle-cell migration. Those two systems seemed quite remote from each other. But like the private eye and the cop who back into each other as they sneak down a dark alley in search of an elusive quarry, Clark and Stern suddenly discovered they were both hot on the trail of the same gene. And not only was the gene a key to such different events as muscle-cell migration and sex-organ development, it also bore a close resemblance to a cancer-causing oncogene called *crk*.



Passing the message. *The protein Ras, encoded by an oncogene, is part of a signaling pathway that is known to begin with an enzyme called a tyrosine kinase—but many of the steps remain to be discovered.*

Their finding, reported this week in *Nature*, illustrates an increasingly common convergence that is taking place in biology these days. Geneticists studying development in both the fruit fly *Drosophila* and the nematode *C. elegans* are finding that the same set of genes trigger not only widely different developmental changes but also cell growth and cancer. The likely benefits of this convergence will be many. The geneticists and biochemists will help each other hop-scotch over problems on the way to a better understanding of the communication that goes on inside cells, and in the process they will see how a common communication machinery can direct events as divergent from each other as normal development is from cancer.

Developmental genetics and cancer research used to inhabit distinct and separate territories, defined by the problems each field addressed. The first sign of a breach of the wall separating the fields came about 10 years ago, when the cancer researchers began to tunnel out of their territory, guided in their quest by the genes they were studying. These proto-oncogenes—the ordinary, noncancerous counterparts of cancer-causing oncogenes—had turned out to be key players in the internal signaling pathways in normal cells. In those pathways, each protein triggers another, which in turn tweaks another, like a chain of people passing a note across a room. The end result may be a change in gene expression or in the activity of key proteins, causing a change in the cell: It may begin to divide as in cancer or in normal growth, or become a neuron or some other kind of specialized cell.

About 5 years ago, geneticists who study development in *Drosophila* and *C. elegans* began to assault the wall from the other side, as they increasingly found their work steering them toward the same signaling pathways. As with the cancer researchers before them, the first revelations came by accident when important developmental genes were cloned, and turned out to be proteins involved in cell signaling. After a few such discoveries, the geneticists began to hone their tools for the express purpose of prying at the signaling pathways and picking out which other genes they may include. In the past year those efforts have begun to pay off in a frenzy of discoveries and publications.

As these rifts in the wall that once kept developmental genetics and cancer research discrete have opened up shaftways of light, the oncogene researchers have become eager for new discoveries. "Genetics has the potential to show up connections that would be very hard [for biochemists] to find," says oncogene researcher Tony Hunter of the Salk Institute. "It can put things in a pathway for you." What Hunter is getting at is that the biochemical approaches favored by oncogene researchers, while they have been successful in identifying signaling proteins and analyzing their biochemical functions—have been less successful at discovering how the proteins fit together as parts of an entire signaling pathway. That's where the geneticists can help. Their methods afford a larger-scale view, with the potential to link together known proteins and fill gaps in the picture with new ones.

The benefits of the relationship flow both ways, according to University of California, Berkeley, geneticist Gerald Rubin. Rubin points out that the findings of his peers may advance knowledge about the system, but they would have little meaning without the biochemical groundwork laid by oncogene researchers that preceded them. To support this point, Rubin recalls that, in 1987, his own group cloned and sequenced a gene called *sevenless*, named because mutations in that gene blocked development of a neuron in the fruit fly eye called R7. When they searched the DNA database for similar genes, they found that *sevenless* resembled a tyrosine kinase—a class of enzyme that acts in signaling pathways by adding phosphate to the amino acid tyrosine on proteins, and is often triggered by hormones or growth factors from outside the cell. But they wouldn't have known that important piece of information, Rubin explains, if oncogene researchers hadn't already turned up many tyrosine kinases, and published their sequences. The oncogene researchers had also learned a lot about the biochemistry of tyrosine kinases and had identified a collection of proteins they phospho-

rylate. But, observes Rubin, they faced "the dilemma of which one of the phosphorylation events is important, and also the dreaded fear that maybe the really important one is too transient or too rare" to show up in biochemical assays.

To address that problem, Rubin and postdocs Michael Simon, Ulrike Gaul, and Graeme Mardon devised genetic methods for finding the genes that are part of the pathway triggered by *sevenless*. They got a handful of mutations, and the first mutants they looked at turned out to have sequences that were similar to known mammalian genes already on the cancer researchers' list of usual suspects: the oncogene *Ras* and the genes for two proteins that interact with the Ras protein, a gene called *Sos* (for *Son of sevenless*) turns out to code for a protein that activates *Ras* by adding a molecule of guanosine triphosphate, and a gene they have named *Gap1*, that codes for a GTPase activating protein (GAP) that seems to inactivate *Ras*. "The fact that we got things that made sense was nice in the beginning," says Rubin, mainly because it showed that the genetic screen was working properly—and not just turning up unrelated genes. But it also added new information, confirming what experiments in cultured cells had suggested—that Ras and its regulators are key components in the tyrosine kinase pathway.

The work coming out of the Horvitz and Rubin labs isn't the only instance of the usual oncogene suspects turning up in some unfamiliar places. Take the case of the *torso* gene, which helps form the head and tail of the early *Drosophila* embryo. *Torso* was cloned several years ago in the laboratory of Christiane Nüsslein-Volhard, at the Max Planck Institute in Tübingen, and it, too, turned out to be a tyrosine kinase. Since then, Helen Doyle, who was a postdoc with Nüsslein-Volhard, and now works with oncogene researcher Michael Bishop at UC, San Francisco (UCSF), has been fleshing out the *torso* pathway. Among the genes she has found are the familiar *Ras* and *Sos*. "The *torso* pathway and the *sevenless* pathway obviously overlap," says Bishop. "They are using some of the very same components to do very different things."

The same-components-different-uses theme extends far beyond *Drosophila*. Caltech geneticist Paul Sternberg and his postdocs Min Han and Jane Mendel and student Raffi Aroian have cloned several genes that control sex organ development in *C. elegans*—and among them are the tyrosine kinase and *Ras* genes. Indeed, looking at how many times some of these oncogenes are surfacing in developmental pathways, Sternberg says, "we have a sense that the pathways are universal. If you have it in nematodes and mammals and *Drosophila*, that's probably the way it works."

Having breached the wall and discovered a remarkable overlap in signal pathways, and the important roles they play in normal development as well as cancer, the geneticists are now pressing forward, hoping for new findings that will fill some of the gaps in the pathways they are piecing together. "The hope," says Berkeley's Rubin, "is to find things that haven't been biochemically identified, or that were known only as biochemical islands, not tied into any pathways."

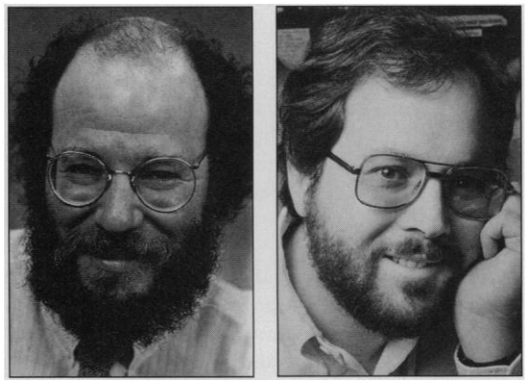
Brand-new biochemical activities haven't yet been found, but UCSF's Bishop says the island-bridging began several years ago, when Harvard geneticist Norbert Perrimon found that a gene downstream of the *torso* tyrosine kinase, called *polehole*, was the same as *raf*, an oncogene that codes for a kinase that phosphorylates proteins on the amino acids serine and threonine. "I remember it vividly," says Bishop. "This was the first example of a connection between these two proto-oncogene products."

Another example of building a new connection is the discovery that Horvitz, Stern, and Clark published this week in *Nature*. The *C. elegans* gene they found, *sem-5*, is related to an oncogene called *crk*, but researchers hadn't yet linked *crk* or its relatives to a tyrosine kinase pathway. *Sem-5* and *crk* share a protein-binding sequence known to oncogene researchers as SH2. The Horvitz finding "places SH2 in a lineage by genetic analysis that is very exciting," says oncogene researcher Harold Varmus, of UCSF. "It suggests a role in a pathway, and that's more than you can say about *crk*."

As for the next part of the program—finding brand new proteins—UCSF's Bishop is optimistic: "The genetics has not revealed a novel biochemical function yet, but it's going to." Indeed, all the labs involved have collections of genes they haven't had the chance to analyze, including some that bear no resemblance to known oncogenes. "We're left hanging on tenterhooks as to whether the remaining genes are something novel," says oncogene researcher Alan Hall of the Institute of Cancer Research in London.

One place Hall and others would like to see some novelty is downstream of Ras in the tyrosine kinase pathway. The biochemical work already done makes it clear that tyrosine kinases are at the head of the pathway, with Ras and the proteins that regulate it a bit further downstream. But beyond Ras lies a mystery: the target molecule Ras operates on. And there the biochemists are looking to the geneticists for a hand. "There is still a great hope that, in *Drosophila* or the worm, the target of Ras, whatever it is, can be identified through genetic means," says Hall.

The search won't end there. For somewhere beyond Ras, the common pathways must diverge to produce different results in different cells. Like a household tool that can be a drill or a screwdriver, depending on which attachment is put on, the common pathway must connect with "customizing" proteins that yield growth in one cell and differentiation in another.



Path-breakers. *Robert Horvitz* (left) *and Gerald Rubin are unraveling the genetics of signal-transduction pathways.*

How that is done is still unknown, but genetics is poised to provide clues. Richard Carthew, in Rubin's lab, for example, has identified a protein in the *sevenless* pathway, called *seven in absentia* (*sina*), that could be such an "attachment" protein. And *sina* has counterparts in other organisms: ex-Rubin postdoc David Bowtell, now at the Florey Institute in Melbourne, Australia, has found a nearly identical gene in mice.

But whether Rubin and Bowtell's proteins help solve the riddle of the divergent pathways, the fact that the usual suspects are turning up in unusual places will no doubt ultimately shed light on the biological crime of cancer. In addition, it will reveal how the same suspects, in different environments, can fill a variety of roles as upstanding citizens of the cell. And, in the end, these two converging lines of evidence will extend researchers' understanding of how the intricate pathways that carry biochemical signals are built. ■ MARCIA BARINAGA

ADDITIONAL READING

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