How *p53* Suppresses Cell Growth

The tumor suppressor *p53* apparently inhibits cell division by eliciting production of a protein that blocks cells' progression through the cell cycle

During the past few years interest in tumor suppressor genes has been building exponentially, and with good reason. Cancer researchers have accumulated convincing evidence that loss of the growth inhibition exerted by these genes plays a major role in cancer. But while the spotlight has been on all known tumor suppressor genes, one of them—*p53*—has garnered most of the attention, because mutations in that gene contribute to the development of up to 50% of all human cancers. Despite all that has been learned about *p53*, however, one critical piece of information has been missing: a solid explanation of just how the normal gene inhibits cell division.

Now, a remarkable confluence of three apparently quite different lines of research appears to have supplied the missing information. Taken together, experiments in several labs show that the protein coded for by *p53* stimulates production of another protein, and that the second protein inhibits key enzymes needed to drive cells through the cell cycle and into mitosis. That picture could explain why the loss of normal *p53* activity leads to uncontrolled cell growth. Besides helping to clarify the causes of cancer, the new work may shed light on aging, since it suggests that the same inhibitory pathway causes senescent cells to stop dividing.

Curt Harris of the National Cancer Institute, who studies *p53* and other gene changes in cancer, describes the finding as "pretty spectacular," an opinion shared by another cancer gene expert, Stephen Friend of Harvard's Massachusetts General Hospital. "I think it's fair to use the word 'spectacular.' This is close to finding the Holy Grail for *p53*," Friend says. He points out that the finding is important from a clinical perspective because it identifies specific targets for new cancer drugs. It might be possible, for example, to design drugs that block cancer cell division by mimicking the inhibitory effects of the *p53*-induced protein on the cell cycle enzymes.

The first firm evidence for a specific biochemical link between *p53* and the cell cycle comes from two groups, one led by Wade Harper and Stephen Elledge of Baylor College of Medicine in Houston and the other by Bert Vogelstein of Johns Hopkins University School of Medicine. Both published their results in the 19 November *Cell*, but the head-to-head publication doesn't mark the end of a race. The two groups came at the work from different directions and were at first unaware of what each other was doing.

The starting point for biochemist Harper, geneticist Elledge, and their colleagues was the cell cycle itself. Over the past few years, work by many groups has shown that passage of cells through the cycle depends on the activity of enzymes known as "cyclin-dependent kinases" (Cdks) because they become active only when they associate with protein partners called cyclins. Harper and Elledge were looking for additional regulatory proteins for the Cdks.

Hypothesizing that a protein would have to bind to the Cdks in order to regulate them, the Harper-Elledge team set out to find the genes for proteins that bind to Cdk2, an enzyme that prepares cells to divide by pushing them out of the first growth phase of the cell cycle into the DNA-synthesizing phase. The protein encoded by one gene they found, which they called *Cip1* (for Cdk-interacting protein 1), proved to be a very good inhibitor of Cdk2 and other Cdks—indicating that it acts as a brake on cell division. That also meant that mutations in *Cip1* might contribute to the abnormal growth of cancer. To ask for advice in investigating that possibility, Elledge called Bert Vogelstein of Johns Hopkins University School of Medicine, who has wide experience in studying the genetic changes in cancer. And that's where Elledge—and Vogelstein—got a big surprise.

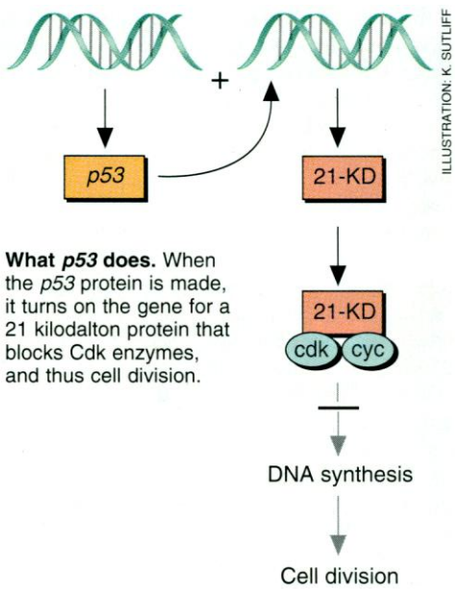


ILLUSTRATION: K. SUTLIFF

What *p53* does. When the *p53* protein is made, it turns on the gene for a 21 kilodalton protein that blocks Cdk enzymes, and thus cell division.

"Steve [Elledge] mentioned almost as an aside that *Cip1* encoded a protein of 21 kilodaltons," recalls Vogelstein, who at the time wasn't thinking particularly about the cell cycle; his focus was on *p53*. But the 21 kilodaltons rang a bell; Vogelstein and Hopkins colleagues Wafik El-Deiry and Kenneth Kinzler had just cloned a gene for a protein with the same molecular weight.

The group was following up on several lines of evidence indicating that the *p53* protein is a transcription factor that acts to turn on the expression of other genes. Such genes would presumably encode proteins that can suppress cell division, and the Vogelstein group had set out to identify and characterize those genes. Their most promising catch: a gene they called *WAF1* (for wild-type *p53*-activated fragment 1). Not only was *WAF1* turned on by *p53*, says, but when put into tumor cells, "it inhibited cell growth much like *p53* itself"—indicating that it might mediate *p53*'s tumor suppressive effects.

It also has a molecular weight of 21 kilodaltons, and in the course of their telephone conversation, Vogelstein and Elledge realized that if *WAF1* and *Cip1* were in fact the same protein, they could explain an awful lot about how *p53* works. They began comparing sequences—and got a match. "It was," says Vogelstein, "the most incredible coincidence that ever happened to me." The two teams hastily re-wrote the discussion sections of their *Cell* papers to include this link between *p53* and the cell cycle.

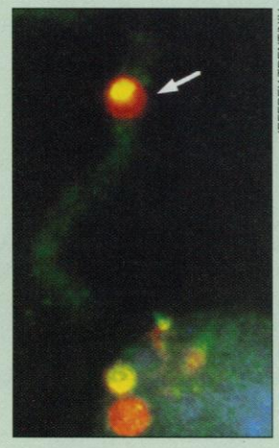
Even though the Harper-Elledge and Vogelstein teams were the first into print, they were not the first to encounter the 21 kilodalton protein. A team led by cell cycle pioneer David Beach at Long Island's Cold Spring Harbor Laboratory also picked up on its possible importance early on. Beach, Yue Xiong, and their colleagues originally discovered the protein in 1992 as part of a binding complex with the Cdks and their associated cyclins in normal cells. And although the Cold Spring Harbor workers didn't clone the gene and get information on its function until this year (the papers are in press at *Nature*), their earlier work did point to a possible link to *p53*.

They found, for example, that while the 21-kilodalton protein is consistently present in the Cdk-cyclin complexes in normal cells,

Gene Defect Identified in Common Hereditary Colon Cancer

In one of a flurry of new developments clarifying the genetic basis of cancer (see accompanying story and articles on pages 1667, 1731, and 1734), scientists have found the gene causing an inherited form of colon cancer, known as hereditary nonpolyposis colon cancer (HNPCC). Although HNPCC is not the only hereditary colon cancer susceptibility to be traced to its genetic root, it is by far the most common. The defective gene is carried by about one person in every 200 and causes up to 15% of all colon cancers.

The discovery, made independently by two teams, one led by Richard Fishel of the University of Vermont Medical School in Burlington and Richard Kolodner of Harvard's Dana-Farber Cancer Center and the other by Kenneth Kinzler and Bert Vogelstein of Johns Hopkins University School of Medicine, should provide a molecular explanation for the widespread genetic damage that leads to the development of this common cancer: The normal gene encodes a protein needed for the repair of damaged DNA. But more than that, it could also lead to the first genetic screen for cancer-susceptible individuals in the population at large.



JEFFREY TRENT/NIH

Hot spot. Staining shows the HNPCC gene location on chromosome 2.

"This is a circumstance where a strong case can be made for presymptomatic DNA testing in the general population," says Francis Collins, Director of the National Center for Human Genome Research at the National Institutes of Health (NIH). He bases this conclusion on the knowledge that not only is HNPCC very common, but that colon cancer can be cured if caught early. So identifying persons who carry the mutant gene is likely to save lives.

Fishel and Kolodner's finding grew out of Kolodner's earlier studies of a particular gene-repair pathway in yeast, known as "mismatch repair" because it removes nucleotides that have paired up with the wrong partners in the DNA double helix and replaces them with the correct ones. About a year ago, Kolodner says, he decided to join with Fishel to identify the equivalent genes in humans. "We thought that these genes would be good candidates for being involved in human diseases," recalls Kolodner, since any mutation that destroys the effectiveness of the repair genes could lead to the accumulation of mutations that could cause diseases, including cancer.

Indeed, barely 6 months after Kolodner and Fishel began looking came a flurry of reports indicating that the defect that causes HNPCC might be in a mismatch repair gene (*Science*, 7 May, pp. 751, 810, 812, and 816). Several groups, including Vogelstein's working in collaboration with Albert de la Chapelle's lab at the University of Helsinki, found that tumors from HNPCC patients as well as some apparently nonhereditary colon cancers show the same kind of genetic instability that had been observed in bacteria and yeast that have mismatch repair mutations. At the same time, the Vogelstein-de la Chapelle team located the probable site of the HNPCC gene—on the short arm of chromosome 2. And with that, the search for the gene moved into high gear.

By then, Fishel, Kolodner, and their colleagues had cloned the human equivalent of the mismatch repair gene *MutS* of bacteria and yeast. When they mapped its location, they got a gratifying result. "It mapped on chromosome 2, really near the locus Bert [Vogelstein] had identified," says Kolodner.

Meanwhile, a large multi-lab team, including Kinzler, Vogelstein, de la Chapelle, and Jeffrey Trent of NIH, was going after the HNPCC gene, using positional cloning techniques to zero in on potential candidates at the chromosome 2 locus. They narrowed the site down to the manageable range of 0.8 kilobases, and within that region they found their candidate—the same human *MutS* equivalent cloned by Fishel and Kolodner.

Still, proving that a candidate gene actually causes a disease requires showing that it is consistently mutated only in those who inherit the disease. Both groups have performed such studies with the human *MutS* equivalent and obtained the hoped-for result, although the Hopkins study was much larger. The patients all "have germline mutations that segregate with the disease," Vogelstein says. (The Fishel-Kolodner results are in the 3 December *Cell* and the Hopkins results will appear in the 17 December *Cell*.)

A further indication that defective gene repair underlies HNPCC comes from studies in which gene repair specialist Paul Modrich of Duke University and the Hopkins team demonstrated that mismatch repair is indeed impaired in the cancer cells and that, presumably as a result, the tumor cell DNA is 100 times more mutable than that of normal cells. Taken together, the results of the two groups leave little room for doubt that they got the right gene.

–J.M.

it is missing from the complexes in several cancer cell lines, including one line known to lack an active *p53* gene. That led them to suggest that *p53* normally regulates the activity of the cell cycle machinery—a suggestion that now appears to be borne out. With regard to cell cycle control, the 21-kilodalton protein "is almost certainly a key effector of *p53*," says Beach.

Still, even Beach's sighting of the 21-kilodalton protein wasn't the first. By the time he talked to Vogelstein, Elledge had learned that his group's *Cip1* is identical to a gene called *sdi1* (for senescent cell-derived inhibitor 1), cloned 2 years earlier by cell biologists James Smith and Olivia Pereira-Smith in their studies of cellular aging. Smith and Pereira-Smith, who are also at Baylor, had been looking for genes that cause cells to go into the senescent decline and lose their ability to divide. The *sdi1* gene seemed to be a good candidate, Smith says. It is much more active in senescent cells than in young cells. And since it inhibits DNA synthesis, it would be expected to block cell division.

But until a few months ago when the Smith team found out about the *Cip1* work, they had no idea how their gene might inhibit DNA synthesis. As a result, Smith says, they were unable to get the work published. Baylor University did, however, apply for a patent on the gene, which was awarded in October, and the researchers now have a paper in press at *Experimental Cell Biology*.

All in all, the new findings are opening up several new lines of investigation concerning the inhibitory protein's possible involvement in aging as well as in cancer. Researchers will want to know, for example, what causes the increased expression of *Cip1/sid1/Waf1* in senescent cells. It will also be important to find out whether mutations in the new gene lead to cancer, as *p53* mutations do, and to search for chemotherapeutic drugs that mimic the cell cycle inhibition of the gene product. With all those questions still to be answered, it seems clear that even though cell cycle and *p53* have just been introduced, they should form a productive partnership.

–Jean Marx