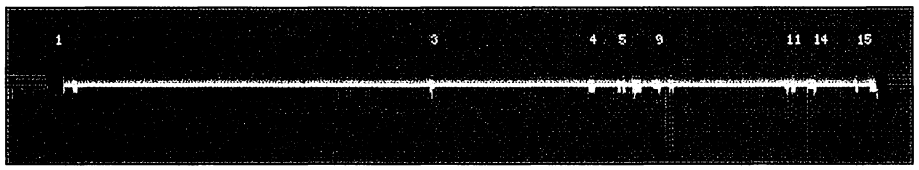
damage to DNA from environmental agents that could otherwise put a cell on the path to cancer. At a symposium here, scientists described initial efforts to find out whether subtle changes in these DNA repair genes can increase a person's cancer risk. If they do, the goal is to identify individuals who are at greater risk so they can take precautions, such as avoiding the sun. So far, researchers have found intriguing hints, but the studies are still very preliminary.



Risk factor? Slight changes in DNA repair genes such as *XRCC1* might, in combination, raise or lower an individual's chance of developing cancer. (SNPs that change protein sequence in pink.)

At least 130 genes are known to code for enzymes that repair DNA, for instance, by fixing such damage as single DNA nucleotide mismatches inflicted by chemical carcinogens, or breaks in DNA strands caused by radiation (*Science*, 16 February, p. 1284). Serious defects in just one of these pathways can be quite dangerous: Xeroderma pigmentosum, caused by various mutations in genes that repair damage caused by ultraviolet rays, can raise a person's risk of skin cancer 1000-fold.

Although such major flaws are very rare, epidemiology studies have found slower overall rates of DNA repair in people with cancer. The explanation, researchers say, may be a constellation of minor mutations in DNA repair enzymes. "There are lots of ways that more subtle variations" could slightly raise a person's risk of cancer, suggests Harvey Mohrenweiser, a biochemist at Lawrence Livermore National Laboratory in California. To get a handle on what mutations exist in the population, Mohrenweiser's team is conducting a systematic survey of common variations in the genome—including tiny, one-base changes called single-nucleotide polymorphisms (SNPs)—in 32 DNA repair genes. Using DNA from an ethnically diverse U.S. population sample, his group is resequencing and comparing the coding regions of these genes in 92 people, enough to find mutations present in at least 1% of the population.

He's finding lots of SNPs. Some are widespread, appearing in half the population, while others are rare. To explore whether these SNPs could affect cancer risk, Mohrenweiser's team has put seven variants of one altered enzyme, Ape1, through cell assays for DNA repair; three were more than 50% slower at mending DNA. At the meeting, Douglas Bell of the National Institute of Environmental Health Sciences (NIEHS) also presented preliminary evidence of an effect in humans: Smokers who had two copies of a certain SNP in the DNA repair gene *XRCC1* had twice as much of a molecular biomarker of DNA damage in their blood as did controls. That suggests that *XRCC1* was not doing its job as well. Bell is part of an NIEHS project studying hundreds of environmental susceptibility genes, from DNA repair genes to many others that metabolize toxicants (*Science*, 24 October 1997, p. 569; see www.genome.utah.edu/genesnps)

Although those studies are interesting, "the real test of whether the SNPs are significant or not," Mohrenweiser says, is whether they are linked to people with disease. In collaboration with Mohrenweiser, molecular epidemiologist Jennifer Hu of Wake Forest University School of Medicine in Winston-Salem, North Carolina, is testing whether people with breast, prostate, or colon cancer have more of certain SNPs than do controls. Some polymorphisms are more prevalent in people with cancer, Hu reported. Two particular SNPs, for example, were 12 times more common in women with breast cancer than in controls and five times more common in people with prostate or colon cancer. But in both her studies and Bell's, other SNPs didn't seem to matter.

Even when they seem to, geneticist Maynard Olson of the University of Washington, Seattle, cautions that associations between disease and SNPs can turn out to be "false positives." He thinks animal studies should be done on potentially important SNPs before researchers expend too much effort on case control studies. Tying SNPs to risks for exposure-related disease "will be a long, slow, and difficult problem," agrees Bell.

–JOCELYN KAISER

CELL BIOLOGY

New Clue to How the Cell Controls Its Proteins

A possible new role for the COP9 signalosome may help explain its function plus that of a recently discovered regulatory process called "neddylation"

Even Leonard Bernstein might have been daunted by the prospect. Somehow, the cell conducts an orchestra with tens of thousands of players—its proteins—ensuring that they come in at the right time and at the right level, harmonize with other players, and stop when their part is finished. Just as it wouldn't do for the French horns to blare their way through an entire concerto, the proteins that drive cell division, say, shouldn't stay active all the time. If they did, the result could be the discordant growth of cancer. Now, two papers published online today by *Science* (www.sciencexpress.org) describe what may be an important new role in this virtuoso performance for a hitherto mysterious eight-protein complex known as the COP9 signalosome (CSN).

Xing-Wang Deng's group at Yale University identified CSN about 7 years ago as a regulator of photomorphogenesis, a developmental response that plants make to light. CSN suppresses the response in the absence of light. Researchers soon learned that the protein complex is widely distributed in animals as well as in plants, but they had few clues to how CSN exerts its effects. The two new papers—one from Deng and his colleagues and the other from Raymond Deshaies's group at the California Institute of Technology (Caltech) in Pasadena—indicate that CSN plays a key regulatory role in a recently discovered process known as "neddylation," at least in some cases turning down a protein by fostering its degradation. CSN may have other biochemical activities as well, however.

One of the major discoveries of the past 15 years was that proteins are regulated not just by addition or removal of small groups such as phosphates, but also by addition of other proteins. The best known of these, ubiquitin, tags proteins for destruction by a large protein complex called the proteasome. In the past few years, cell biologists have found more of these protein tags, including one called Rub1 or NEDD8—hence the term neddylation. Researchers are just beginning to understand what role the addition and removal of NEDD8 plays in protein regulation, but one emerging idea is that NEDD8 indirectly influences protein destruction, possibly through the ubiquitin system. Deng's and Deshaies's teams' work indicates that CSN is a partner in this activity.

The researchers found that CSN removes NEDD8 from an enzyme called SCF,

CREDIT: UNIVERSITY OF UTAH GENOME CENTER

which adds ubiquitin groups to other proteins, and they demonstrated that this has physiological consequences. Specifically, when CSN doesn't remove NEDD8, plants don't respond normally to auxin hormones, which control branching, root growth, and other developmental processes. Ubiquitin researcher Keith Wilkinson of Emory University School of Medicine in Atlanta, Georgia, describes the findings as "exciting. ... We are beginning to understand what molecules might be regulated by CSN." And although this work has pinned down a physiological role for CSN-mediated removal of NEDD8 only in plants, there are hints that it may have much more general importance, as CSN components interact with a variety of animal and plant cell proteins.

Deshaies and his colleagues stumbled across the function of CSN while studying SCF. The Caltech workers used two of the four protein subunits that make up SCF to fish for other proteins that associate with that enzyme in mouse cells. To their surprise, they found that one SCF subunit, which goes by the name CUL1, pulled out all eight CSN proteins. Previous work had suggested that CSN might be involved in protein degradation, because it is composed of proteins that resemble those that make up a portion of the proteasome, the destroyer of ubiquitin-tagged proteins.

Still, suspecting that CSN is somehow involved in protein degradation and proving it are two different things. After what Deshaies describes as "a number of blind alleys," he came across a report by Anthony Carr and his colleagues at the University of Sussex in Brighton, United Kingdom, who found that CSN is present in the fission yeast (*Schizosaccharomyces pombe*), an organism much more amenable to genetic studies than the mouse. Deshaies and his colleagues went on to examine a mutant yeast strain produced by Carr that lacks the gene for one of the CSN subunits and thus lacks a functional CSN.

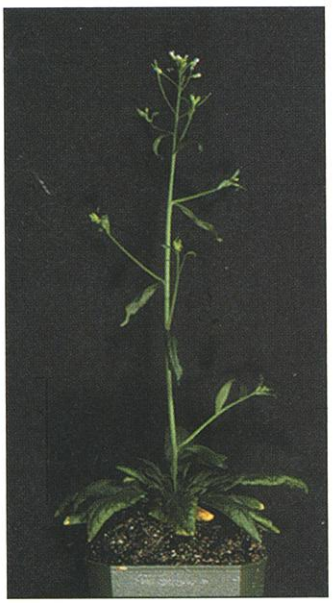
The SCF subunit CUL1 is one of the few cellular proteins known to be tagged by NEDD8. That modification stimulates SCF to add ubiquitin to other proteins. In normal cells, Deshaies says, only a small fraction of CUL1 is neddylated. But "in the signalosome mutant, 100% of the molecules have this modification." That suggested that loss of CSN function somehow prevented removal of NEDD8 from CUL1.

Ultimately, Deshaies and his colleagues traced the "deneddylating" activity directly to CSN. The most definitive demonstration came when they mixed CSN purified from pig cells with purified neddylated CUL1. The CUL1 was promptly deneddylated. The activity "has to be associated with the signalosome," Deshaies concludes. However, he hasn't ruled out the possibility that some other tightly bound protein that purifies along with CSN removes NEDD8 from CUL1.

Deng's team then linked the CSN activity that the Deshaies group found to a specific physiological role. In collaboration with four groups including Deshaies's, they turned to the plant *Arabidopsis thaliana*. The researchers didn't want to knock out CSN completely, because the plants would die well before maturity. Instead, the team created a mutation that simply reduced CSN activity.

The resulting plants looked very much like plants that have lost their responsiveness to auxin hormones. For example, auxin suppresses the development of secondary flower-bearing branches, and the mutant plants have roughly three times as many such branches as normal plants have. The researchers also found that the roots of mutant plants are resistant to auxins.

That provided another clue. Mark Estelle of the University of Texas, Austin, a coauthor of the Deng paper, and his colleagues had previously shown that auxin responses in *Arabidopsis* depend on the ability of one of that organism's SCFs to ubiquitinate a suite of proteins normally turned on by the hormones. When those proteins aren't ubiquitinated, they accumulate; that, in turn, causes auxin resistance.



Suppressed signal. A mutant *Arabidopsis* plant with reduced CSN activity (*left*) is resistant to the plant hormone auxin as indicated by its having more secondary branches than a normal plant (*above*).

CREDIT: C. SCHWECHHEIMER *ET AL.*

Together, these findings suggested that CSN might help control the degradation of the auxin-induced proteins. Soon Deng and his colleagues found that abnormally high amounts of one of the proteins do in fact build up in their CSN-deficient plants—an indication that CSN normally acts in some fashion to bring about degradation of the protein. Further work by the team indicated that the buildup might be due to a loss of CSN's ability to remove NEDD8 from the CUL1 subunit of the *Arabidopsis* SCF. For example, the researchers found that neddylated CUL1 accumulates in the mutant plants just as it does in the mutated fission yeast strain.

Stefan Jentsch, who studies ubiquitination and neddylation at the Max Planck Institute for Biochemistry in Martinsreid, Germany, describes the Deng team's work as "intriguing. [They] convincingly show that CSN is required for normal auxin response and that it promotes degradation of a specific protein regulator."

Still, many questions remain. One is just how CSN's proposed deneddylation of CUL1 leads to increased protein degradation. At first glance the removal of NEDD8 might be expected to decrease protein degradation by inhibiting SCF's ability to attach ubiquitin to proteins. "It's a complete surprise. It's counterintuitive," says Deshaies. One possibility, Deng suggests, is that cycling between NEDD8 addition and removal might be necessary for normal SCF function in adding ubiquitin to proteins.

Also unclear is how broad a role CSN plays in the cell. In plants, it works in both photomorphogenesis and auxin responses and in other systems as well. CSN may regulate many other proteins, Deshaies predicts. Other researchers have found that CSN subunits interact with a variety of proteins, and Deshaies and his colleagues have found increased neddylation of other, as yet uncharacterized, proteins in CSN mutants.

And neddylation may not be CSN's only biological activity. Wolfgang Dubiel's group at Humboldt University in Berlin has evidence that the signalosome can attach phosphate groups to proteins. Most recently, the researchers reported in the 2 April issue of the *EMBO Journal* that when CSN phosphorylates the tumor suppressor protein p53, it targets the protein for degradation by the ubiquitin system. Because CSN contains eight subunits, Wilkinson says that "it wouldn't surprise me at all" if the signalosome had more than one activity. Indeed, CSN and its activities in neddylation and elsewhere should give cell biologists plenty to work on in the next few years. **–JEAN MARX**