CONTINUED FROM PAGE 221

effort so that we spend it wisely."

Foundation directors are not sitting behind their desks anxiously waiting for advice from researchers about how to reduce their philanthropy's assets, however. "Boy, am I not soliciting proposals," said Gates Sr. last June, echoing a sentiment voiced by several foundation heads. "If somebody heard that the director of the William H. Gates Foundation was trying to figure out a way to spend money, my life would be really rendered almost inoperative. I'd be overwhelmed with people." Then again, Gates does have to figure out how to spend what now amounts to $2.3 million a day. So it may pay, literally, for researchers to watch closely as Gates and other foundations that have more money than anyone would have imagined a few years ago articulate visions for their futures. –JON COHEN

DISCLOSURE

By way of full disclosure, it should be noted that various activities of the AAAS (*Science*'s publisher) are supported by grants from several organizations mentioned in the preceding articles, including the Howard Hughes Medical Institute, the Pew Charitable Trusts, the Burroughs Wellcome Fund, and the Wellcome Trust. In addition, the Sloan Foundation provided funding last year for a book by author Jon Cohen.

CELL BIOLOGY

A New Finger on the Protein Destruction Button

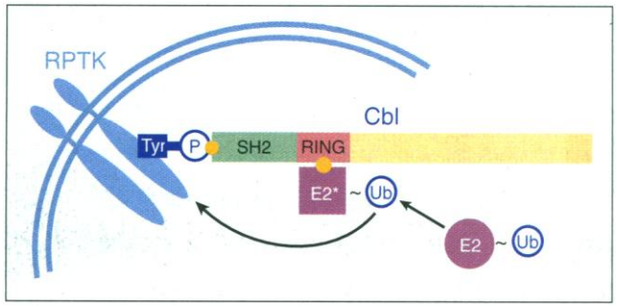
A protein motif called the RING finger helps add ubiquitin to proteins, thereby marking them for the cellular trash heap

To make an omelet you have to break a few eggs, and to keep a cell healthy you have to destroy some proteins. Recent evidence has shown that the timely eradication of proteins that drive cell division is vital to keeping normal growth from turning into runaway malignancy. In the biological equivalent of putting trash bags by the curb, cells tag proteins for elimination by attaching a small protein called ubiquitin. The tagging occurs in steps, with one kind of enzyme binding to the condemned molecule and another ferrying the ubiquitin label to the target. In the past few months, researchers have identified a molecular motif that marries these two kinds of proteins so that the tagging can take place.

Fittingly, it's called the RING finger, an evolutionarily conserved structure found in more than 200 proteins, in which two loops of amino acids are pulled together at their base by eight cysteine or histidine residues that bind two zinc ions. A new crop of results, including those reported on page 309 by Tony Hunter's team at The Salk Institute in La Jolla, California, show that several RING finger proteins participate in ubiquitination. Among these are two proteins that play roles in cell growth control: Cbl, which can cause cancer when it is mutated, and BRCA1, the breast cancer susceptibility gene.

But given the large number of proteins that contain RING fingers, these discoveries may be just the tip of the iceberg. "Many [of these proteins] have been implicated by genetics as participating in some process or other, but without any clear mechanistic insight as to how they are acting," says cell biologist Ray Deshaies of the California Institute of Technology (Caltech) in Pasadena. The new results are likely to touch off a wave of research on how these proteins might regulate cell activities and also exploration of how the chain of protein destruction might be restored in some cases to halt the growth of cancer cells.

Hunter and his colleagues, postdoc Claudio Joazeiro, and cell biologist Yun-Cai Liu at the La Jolla Institute for Allergy and Immunology in San Diego, made their observations while following up on recent work on the protein Cbl. A genetic analysis done in Paul Sternberg's lab at Caltech had revealed that the version of Cbl found in the roundworm *Caenorhabditis elegans* is necessary for turning down the activity of a growth-promoting protein, the receptor for epidermal growth factor (EGFR). And last year, groups led by Hamid Band at Harvard, Yosef Yarden at the Weizmann Institute of Science in Rehovot, Israel, and Richard Stanley at Albert Einstein College of Medicine in the Bronx reported evidence that Cbl down-regulates growth factor receptors by helping to ubiquitinate them, marking them for destruction.



Missing link. Cbl helps tag a membrane-bound receptor for destruction by bringing it together with a ubiquitin-loaded E2.

To pinpoint the parts of the protein required for ubiquitination, Yarden's group took a cue from two known Cbl mutants that cause lymphoma cancers in mice. Both are missing chunks of the protein's RING finger, suggesting that it is vital to the protein's normal function. They report in the 6 August issue of the *Journal of Biological Chemistry* that one of these natural mutations, as well as a lab-made mutation, both prevent Cbl from ubiquitinating the EGFR in cells.

Hunter's team went further to parse out Cbl's exact role in ubiquitination, which requires three different kinds of proteins. One called E1 activates ubiquitin, an E2 protein temporarily holds the activated ubiquitin, and the E3 enzymes bind the target and guide the transfer of the ubiquitin to it. Because Cbl binds ubiquitination targets such as the growth factor receptors, Hunter and others in the field wondered whether it might be an E3.

To investigate that hunch, and to test the RING finger's specific role, Joazeiro isolated Cbl's RING finger and tested its ability to trigger ubiquitination in a test tube. He engineered bacteria to manufacture just the RING portion of Cbl, linked for production purposes to a bacterial protein. He then mixed the hybrid protein with ubiquitin, E1, and E2 to see if it would function as an E3.

It worked. The researchers detected ubiquitination of proteins in the reaction, and mutations of key amino acids in the RING finger abolished the effect, pegging the RING finger as essential. What's more, Liu found that the RING finger directly binds to the E2 enzyme. That proves "that the RING finger domain of this protein is capable of recruiting the E2 component," says Band, "which is critical, because that begins to provide a basis for how Cbl might enhance ubiquitination."

But getting ubiquitination of one of Cbl's known targets, the platelet-derived growth factor receptor (PDGFR), required a bigger piece of Cbl, containing the RING finger

CREDIT: C. JOAZEIRO, MOLECULAR BIOLOGY AND VIROLOGY LABORATORY/THE SALK INSTITUTE FOR BIOLOGICAL STUDIES

plus a motif called an SH2 domain. SH2 binds to a feature common to many activated receptors including EGFR and PDGFR: a tyrosine amino acid bearing a phosphate group. "Our model is that the SH2 domain targets the RING finger to the receptor ... via a [phosphorylated tyrosine], and now the associated E2 can ubiquitinate the receptor," Hunter says. That marks the receptor for destruction, which "is really important," he adds, because "you don't want the receptor to go on signaling forever." Because phosphorylated tyrosine is found on many other growth regulatory proteins, Hunter suggests that Cbl may help ubiquitinate these proteins as well.

And it may be only one of many RING finger proteins controlling cell processes in this way. In the past, E3 enzymes have mystified cell biologists, because they showed no obvious sequence similarities in spite of their common function. But three papers in April, and one in June by Deshaies's team, revealed a common thread, a RING finger protein, in three different E3 complexes (*Science*, 23 April, p. 601). That discovery "all of a sudden crystallized things," says Deshaies. It suggested that "there actually may be much more of a relationship between all these E3s than was previously anticipated."

What's more, the findings suggested that other RING finger proteins act as E3s, a possibility that Allan Weissman's team at the National Cancer Institute was already pursuing. In the 28 September issue of the *Proceedings of the National Academy of Sciences*, they report that they tested seven RING finger proteins and found that they all trigger ubiquitination in the test tube.

One is the product of the *BRCA1* gene, which, when mutated, increases the risk of breast cancer. BRCA1 seems to take part in DNA repair, but despite long study, its exact role isn't known, says Frank Rauscher, who studies protein signaling at the Wistar Institute in Philadelphia. But he notes, "There is ample evidence to suggest that DNA repair is regulated exquisitely by the ubiquitin system." The new finding opens the possibility that BRCA1 helps ubiquitinate DNA repair proteins.

That is just one of the new insights likely to be fueled by the current work, Rauscher says, noting that other RING finger proteins have roles—ill-defined so far—in other important cell processes such as gene expression and X-chromosome inactivation. "What are these other RING fingers doing?" he asks. "There are a huge number of questions that can now be addressed using this new function of the RING finger."

–MARCIA BARINAGA

CELL BIOLOGY

First Components Found for Key Kidney Filter

The discovery may help researchers understand normal kidney function and provide new targets for therapies for certain types of kidney diseases

When a baby named Toni was born in Helsinki, Finland, in 1988, his doctor, Christer Holberg, soon found that his kidneys were malfunctioning dangerously: They were leaking proteins into his urine, causing him to lose massive amounts of vital molecules. To live, Toni would need a kidney transplant. He got it, and also lent a hand to research that should help scientists understand both the normal functioning of the kidney and how that functioning breaks down in disease.

Last year, by studying Toni's family and others with the same condition, a team led by molecular biologist Karl Tryggvason of the Karolinska Institute in Stockholm, Sweden, identified the gene at fault in the disease, congenital nephrotic syndrome. Since then, the researchers have shown that nephrin, the gene's protein product, is a major component of the filter in the kidney that keeps vital proteins from leaving the body via the urine. It is not the only protein needed to build the kidney's filter, however. On page 312, a group led by immunologist Andrey Shaw at Washington University School of Medicine in St. Louis, Missouri, describes a second protein, CD2AP, that anchors nephrin to adjacent cells to form the filter.

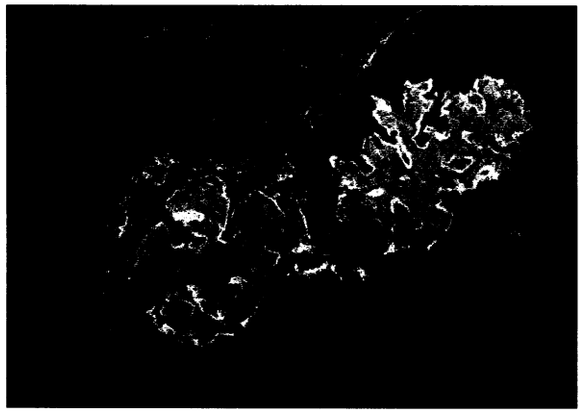
Kidney specialists are hailing these discoveries, because they may help them understand more than just relatively rare cases of congenital kidney disease. The kidney filter, known as the slit diaphragm, can also break down later in life, often as a complication of common diseases such as high blood pressure, diabetes, and lupus, a disorder of the immune system in which the body attacks its own tissues. Such damage can ultimately lead to kidney failure and death as the body loses its ability to clear its waste properly.

"These are terribly exciting breakthroughs," says nephrologist Jared Grantham of the University of Kansas Medical Center in Kansas City, Kansas. "We are drawing a molecular map of a critical barrier in the kidney." That may, he adds, provide potential targets for therapies for a class of kidney diseases—those characterized by protein in the urine—that accounts for about half of the 280,000 cases of end-stage kidney disease in the United States alone.

For years, researchers have viewed the slit diaphragm as the ultimate barrier that prevents proteins from leaking into the urine from capillaries in the tiny globes of tissue called kidney glomeruli where the urine is first produced. The diaphragm forms in the gap between long projections, or "foot processes," that extend from cells called podocytes and wrap around the glomerular capillaries.

In 1974, electron micrographs made by Richard Rodewald and Morris Karnovsky of Harvard Medical School revealed that the diaphragm is a zipperlike formation of molecules, with a groove (the slit) down the middle. The spaces between the prongs of the zipper were just the right size for the filtering job: too small to let proteins leave the capillaries, but big enough to allow sugar and water through. But for decades, no one could identify the molecules that make up the slit diaphragm. So in 1989, Tryggvason's team set out to find a slit protein the hard way—by tracking down the gene coding for it.

Children with congenital nephrotic syndrome consistently show problems with their slit diaphragms, seen by examining tissue from kidney biopsies with an electron microscope. So Tryggvason reasoned that the defective gene might code for a part of the



Defective filters. In CD2AP knock-out mice, the mesangial cells (green) of the kidney glomeruli expand relative to the podocytes (red). Blue staining marks various cell nuclei.

CREDIT: J.H. MINER, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE