The cleanup protein has been known to be present in all higher organisms, but now researchers are discovering that it has a hand in everything from directing protein traffic to regulating gene activity

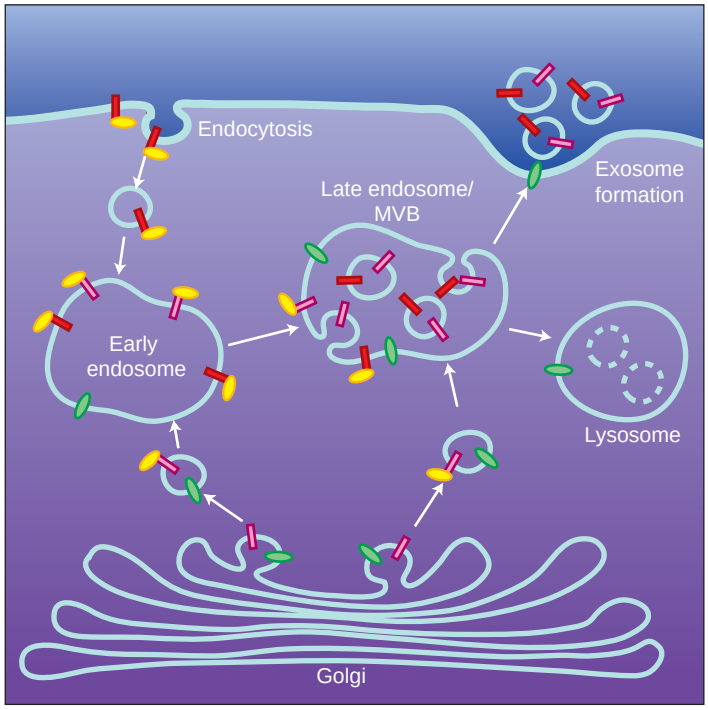
Ubiquitin Lives Up To Its Name

A small protein called ubiquitin is turning out to be the Clark Kent of cell biology. Like Superman's alter ego, ubiquitin has long been regarded as worthy but somewhat dull, a player in the cast of characters that carry out housekeeping functions for the cell. But recent findings are beginning to reveal it as a kind of superhero, performing feats that few suspected.

Early work showed that ubiquitin, which was discovered in the mid-1970s, is part of the cell's janitorial services. It binds to other proteins, tagging them for destruction by a large multiprotein complex called the proteasome. This kiss of death eliminates damaged proteins, an essential job but perhaps not one to catch the eye of Lois Lane. But ubiquitin-mediated protein disposal soon turned out to have a more glamorous role: helping regulate such key cellular processes as the cell division cycle. Now researchers are finding that ubiquitin's functions go far beyond even these crucial activities.

Recent work, much of which was on display at a meeting* last month, shows that ubiquitin plays traffic controller as well as janitor. Ubiquitin tagging directs the movement of important proteins in the cell, determining, for example, whether they end up on the cell membrane or in an internal vacuole, where they are destroyed without the proteasome's help. "The whole aspect of ubiquitin-mediated [protein] trafficking inside the cell is brand-new," says cell biologist Annette Boman of the University of Minnesota, Duluth. "It was really a surprise to me and to pretty much everyone else in the field as well."

Other work indicates that ubiquitin and related proteins play direct roles in controlling the machinery that brings about gene expression. The multipurpose molecule also helps regulate the many signaling pathways that control the cell's responses to environmental and other changes. Indeed, the meeting co-organizers, Cecile Pickart of Johns Hopkins University in Baltimore and Linda Hicke of Northwestern University in Evanston, Illinois, say that ubiquitin's actions in the cell might be as pervasive—and as important—as those of the well-known regulator phosphate, which controls the activities of thousands of proteins.



Traffic signal. A ubiquitin tag (yellow ovals) tells proteins, whether on the outer membrane or newly synthesized and in the Golgi apparatus, to move into the endosome and the multivesicular body (MVB).

The new appreciation of ubiquitin's multiple roles has medical implications. It turns out, for example, that ubiquitin helps turn off the cell's responses to growth factors; without this safeguard, the uncontrolled cell growth of cancer might result. And researchers are also finding that certain viruses that bud from the cell surface, including Ebola and HIV, do so by commandeering the same ubiquitin-dependent transport machinery used for protein trafficking in the cell.

Entry and sorting

One of the most advanced lines of work on ubiquitin's newfound powers deals with the protein's role in directing protein movements. Some puzzling observations in the mid-1990s provided the first clues that ubiquitin might somehow be involved in bringing membrane-bound proteins into the cell.

At the time, cell biologists suspected that some membrane proteins are ubiquitinated and degraded in the standard fashion by the proteasome. But they found that ubiquitin is also added to proteins, including growth factor receptors, that meet a different fate. Cells turn these receptor responses down or off by bringing the receptor into the cell in tiny membranous sacs called endosomes, which form when the external membrane bulges into the cell and buds off. Once inside, the endosome cargo is either directed to vacuoles called lysosomes for degradation or recycled back to the cell membrane.

The finding raised suspicions that the ubiquitin tag might be the signal for internalizing the receptors, but as Pickart recalls, "one thing [we thought] we knew for sure is that ubiquitin had nothing to do with the lysosome." Direct proof that it does came a few years later, providing what Pickart calls "a satisfying reverse of course."

One series of key experiments came from Hicke, then working with Howard Riezman of the University of Basel, Switzerland, on a yeast cell receptor called Ste2p, which responds to one of the factors that controls yeast mating. The researchers showed that Ste2p becomes ubiquitinated when it binds the mating factor and that as a result, it is taken into the cell and degraded by the lyso-

ILLUSTRATION: ADAPTED FROM W. SUNDQUIST/UNIV. OF UTAH

* The meeting, "Nontraditional Functions of Ubiquitin and Ubiquitin-like Proteins," was sponsored by the American Society for Cell Biology and held from 11 to 14 August in Colorado Springs.

some. Replacing the amino acid where ubiquitin latches onto Ste2p with a different one eliminated both the ubiquitination and internalization of the receptor. Studies with other mutants confirmed that the resulting degradation takes place in the lysosome.

Subsequent work also provided an explanation for how the cell might determine which ubiquitinated proteins are to be degraded by the lysosome and which by the proteasome. Proteins headed for the proteasome are tagged with a string of at least four ubiquitins, whereas Ste2p was marked with only one. Since then, numerous researchers have shown that ubiquitin tags membrane proteins for internalization in endosomes in both yeast and more advanced organisms, including mammals.

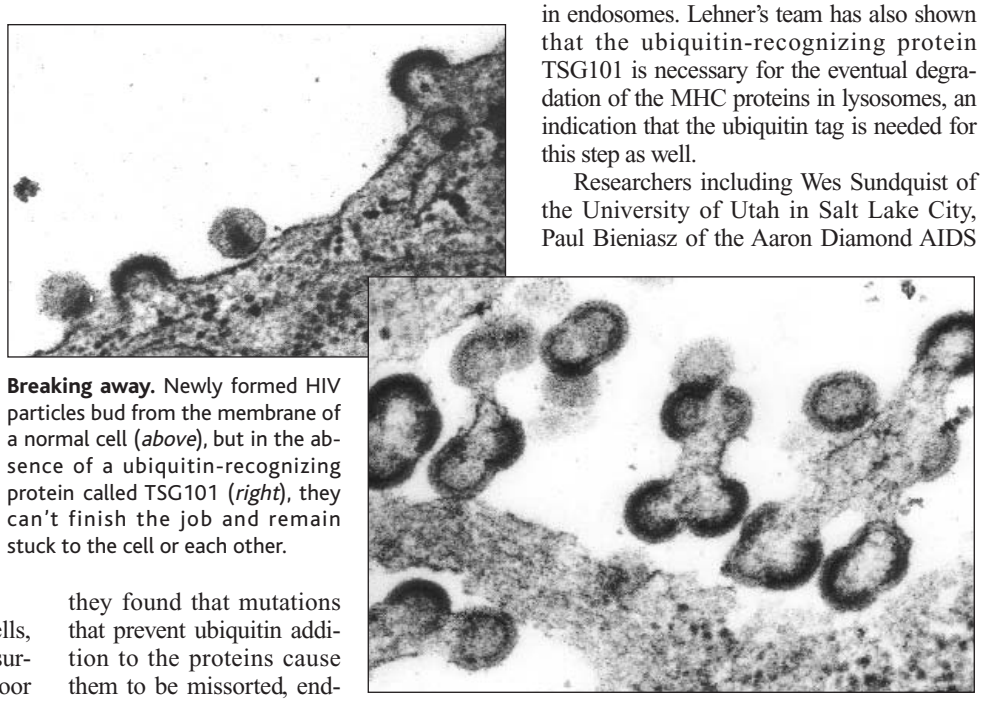
More recent work, reported within the past year or two, has uncovered a broader role for ubiquitin in directing protein traffic within the cell. Not only does it mark membrane proteins for internalization, it also helps determine whether newly synthesized proteins get to the membrane in the first place. Proteins destined for the cell surface are separated from others at the end of a series of membranous compartments collectively called the Golgi apparatus. Ubiquitin has now been implicated in this decision. For example, at the meeting, Chris Kaiser of the Massachusetts Institute of Technology described evidence for the idea, obtained in studies on a yeast protein called Gap1 that carries amino acids across the yeast cell membrane.

Gap1 is made all the time in yeast cells, but it is normally transported to the cell surface only when yeast are growing on a poor nitrogen source. If there's ample nitrogen, Gap1 moves into the endosome and from there possibly into the lysosome for degradation. Kaiser and his colleagues found that mutations that increase ubiquitin addition to Gap1 cause it to move into the endosome even when yeast is growing on a poor nitrogen source. Conversely, mutations that decrease Gap1 ubiquitination result in its being transported to the cell membrane when yeast cells have an ample nitrogen supply.

"Ubiquitin tagging can be used as a sorting signal," Kaiser says. He adds that a system in which cells continuously synthesize Gap1, and then use ubiquitin to determine its fate, might enable yeast to respond more rapidly to changes in nitrogen availability than would be possible if they had to fire up Gap1 synthesis from scratch.

Ubiquitin tagging also helps sort proteins at a later stage in the protein transport pathway, determining which go to the lysosome for degradation and which stay in the endosomal membrane for possible recycling. Earlier studies had revealed that this sorting occurs when the endosome membrane buds inward, forming smaller vesicles inside the larger one. This so-called late endosome or multivesicular body (MVB) then fuses with the lysosome and dumps the small vesicles into the lysosomal interior where they can be degraded.

About a year ago, two teams, one including David Katzman, Markus Babst, and Scott Emr of the University of California, San Diego (UCSD), and the other including Fulvio Reggiori and Hugh Pelham of the U.K. Medical Research Council's Laboratory of Molecular Biology in Cambridge, reported evidence that ubiquitinated proteins end up in the vesicles inside the MVB. For example, they found that mutations that prevent ubiquitin addition to the proteins cause them to be missorted, ending up in the endosomal membrane rather than the lysosome. Ubiquitin tagging is "a beautiful mechanism for separating those [proteins] that recycle and those that don't," Emr says.



Breaking away. Newly formed HIV particles bud from the membrane of a normal cell (*above*), but in the absence of a ubiquitin-recognizing protein called TSG101 (*right*), they can't finish the job and remain stuck to the cell or each other.

In addition, the Emr team has identified a multiprotein complex, called ESCRT-I (for endosomal sorting complex required for transport), that apparently recognizes ubiquitinated proteins in the endosome and somehow shepherds them into the MVB interior vesicles. The ESCRT-I component that achieves this recognition is a protein called Vps23 in yeast and TSG101 in mammals. In a particularly interesting twist on the protein-trafficking theme, researchers have recently tied TSG101 to certain key steps in viral infectivity.

Appropriated by viruses

Virologists have known for years that viruses often subvert immune attack on the cells they infect by removing major histocompatibility complex (MHC) proteins from the cell surface. MHC proteins evoke immune responses by displaying fragments of foreign antigens, including those from viruses; thus their loss results in a weakened immune attack. In work described at the meeting and also in the 15 May issue of *EMBO Journal*, Paul Lehner of the Cambridge Institute for Medical Research in Cambridge, U.K., and his colleagues showed that the virus responsible for Kaposi's sarcoma is involved in the down-regulation of MHC proteins in two ways.

They found that a viral protein called KK3 promotes the addition of ubiquitin to MHC class I proteins on the cell membrane, fostering their movement into the cell interior in endosomes. Lehner's team has also shown that the ubiquitin-recognizing protein TSG101 is necessary for the eventual degradation of the MHC proteins in lysosomes, an indication that the ubiquitin tag is needed for this step as well.

Researchers including Wes Sundquist of the University of Utah in Salt Lake City, Paul Bieniasz of the Aaron Diamond AIDS Research Center and Rockefeller University in New York City, and Carol Carter of the State University of New York, Stony Brook, have shown that ubiquitin and TSG101 play a different, but related, role in the life cycle of RNA viruses, including HIV and Ebola, that exit infected cells by budding from the cell membrane.

When this budding is about to occur, the RNA-containing core of the virus makes its way to the outer cell membrane, where viral envelope proteins have already been incorporated. The membrane then bulges outward until the virus is released, ensconced in its envelope. Sundquist and the other researchers have found that completion of this budding requires TSG101. It apparently recognizes the viral envelope protein gag, which is ubiquitinated and also carries a particular four–amino acid sequence needed for the interaction. TSG101 presumably then draws other proteins needed for budding to the cell membrane. As UCSD's Katzman noted at the

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meeting, "the virus is usurping the [MVB] budding system to its own ends."

The findings also raise the possibility of targeting TSG101 or other components of the budding machinery with antiviral drugs. "You can envision that inhibitors would give fairly broad antiviral activity," Sundquist says. He cautions, however, that at this early stage of the work, "we have no idea how toxic such an inhibitor will be."

Central command

Protein trafficking takes place in the cytoplasm, but ubiquitin's range has recently been extended to the nucleus. Several teams have linked the protein to various components of the machinery that carries out the first step in gene expression: copying the DNA's code into messenger RNA. Previous work had shown that ubiquitin can control gene activity indirectly by tagging for destruction various proteins involved in gene expression. But new findings suggest that it also has a direct role in determining whether genes are turned on or off.

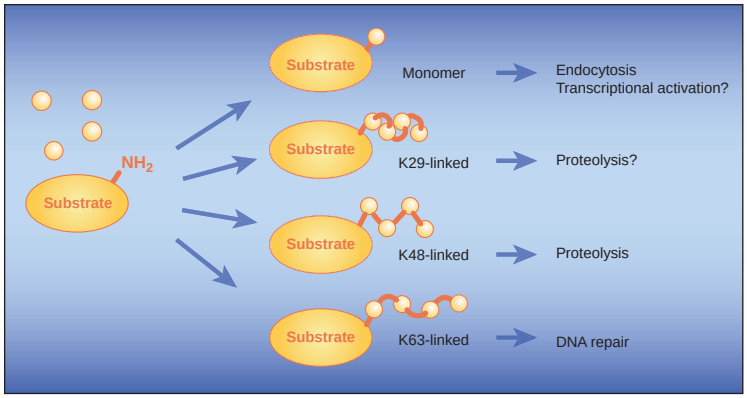
Clues that this might be happening date back 25 years to when ubiquitin was discovered. One of the first proteins found to be modified by ubiquitin was a histone: a member of a family of proteins that packages DNA into chromatin. At the time, that was puzzling, says David Allis, a transcription researcher at the University of Virginia, Charlottesville. Histones were thought to be "rock-stable" and therefore not susceptible to ubiquitin-mediated degradation. "For 25 years, it remained unclear what [ubiquitin's] role was in chromatin. We were really scratching our heads," Allis remarks.

It now looks as if ubiquitination could contribute to the chromatin remodeling that helps regulate gene activity. In early 2000, for example, in what Allis describes as "beautiful" work, Mary Ann Osley, now at the University of New Mexico Health Sciences Center in Albuquerque, and her colleagues showed that histone H2B in yeast is ubiquitinated by an enzyme called Rad6 or Ubc2 (*Science*, 21 January 2000, p. 501).

More recent evidence from the Osley group indicates that this modification aids the uncoiling of the chromatin necessary before a gene can be transcribed. A mutation that prevents ubiquitin addition to the histone partially inhibits transcription of two genes called *Suc2* and *Gal1*. The effect was much magnified if the researchers also prevented histone acetylation, a chemical modification known to facilitate gene transcription—an indication that the two modifications work together.

In other circumstances, ubiquitin addition by Rad6 might be involved in gene silencing instead of gene activation. Addition of a methyl group to histone H3 leads to the inactivation of certain genes, and earlier this year, Zu-Wen Sun, a postdoc in the Allis lab, and independently Ali Shilatifard of St. Louis University School of Medicine and his colleagues showed that ubiquitin has to be added to histone 2B by Rad6 before the H3 histone can acquire its methyl group. The findings "give the chromatin field a whole new modification to worry about," says Allis.

Meanwhile, researchers studying transcription factors—proteins that interact with DNA to alter gene expression patterns—are taking a new look at a modification that seemed to be well understood. Ubiquitination is an established way to tell the cell to eliminate short-lived, or unstable, transcription factors. But it might do much more than that.



Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

A year or two ago, William Tansey and his colleagues at Cold Spring Harbor Laboratory (CSHL) in New York state picked up on an odd coincidence: The region in the transcription factors that serves as a signal for ubiquitin addition overlaps with a region required for activating gene transcription. That overlap, Tansey says, is "found in just about every unstable transcription factor."

Last year, the CSHL group provided evidence that this overlap has functional significance. The researchers showed that for at least one transcription factor, addition of ubiquitin to the site is needed for it to turn on gene expression. Ultimately, the same ubiquitin might serve as a signal for degradation. Cell biologist Joan Conaway of the Stowers Institute for Medical Research in Kansas City, Missouri, describes these findings as "most intriguing, but not yet well understood."

For instance, researchers don't yet know what enzyme puts ubiquitin on transcription factors, although Joan Conaway and Ronald Conaway of Stowers and their colleagues have a possible clue. Many proteins must cooperate to bring about gene expression, and the researchers found that a multiprotein coactivator of transcription called Mediator contains a component that might be involved in recruiting ubiquitin-adding enzymes to the transcription machinery.

Also unclear is exactly how ubiquitin addition promotes transcription factor activity, but it might help recruit some of the other proteins needed for gene activity to the target genes. For example, Stephen Johnston, Thomas Kodadek, and their colleagues at the University of Texas Southwestern Medical Center in Dallas have found that a portion of the proteasome itself is involved in transcription. Still, all of this remains to be established. "We have a lot of possibilities for what [ubiquitination] might be doing [in transcription], and we're in the early days of trying to figure this out," Joan Conaway says.

In addition to ubiquitin's roles in protein trafficking and gene transcription, the protein and its relatives are turning up as possible regulators of many of the cell's signaling pathways. For example, at the meeting, Ajay Chitnis of the National Institute of Child Health and Human Development in Bethesda, Maryland, reported evidence implicating ubiquitination in regulation of a major developmental pathway called Notch. And Rick Firtel of UCSD described a role for a ubiquitin relative called SUMO in controlling the response of the slime mold *Dictyostelium* to chemical signals. Echoing a refrain sounded by many others, Firtel said, "We didn't start out studying ubiquitin but then found ourselves right in the middle of the research."

Indeed, Northwestern's Hicke says that ubiquitin regulation might be even more widespread and versatile than regulation by phosphate. As proteins, she notes, ubiquitin molecules can be joined to other proteins in a variety of ways, either individually, as apparently happens when proteins are marked for uptake by endosomes, or in chains, as occurs in tagging for destruction by the proteasome. In addition, some half-dozen related proteins, such as SUMO, are turning up as regulators of cell activities. "The variety of [possible] modifications is virtually endless," Hicke says. Whether or not ubiquitin turns out to be superpowerful everywhere it raises its head, it certainly seems poised to keep cell biologists in thrall. **–JEAN MARX**

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