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Nobel Committee Tags Ubiquitin for Distinction

Essay

Alfred L. Goldberg*
Department of Cell Biology
Harvard Medical School
Boston, Massachusetts 02115

It must be very difficult to serve on the committee to select Nobel Prize winners—to have to select one or two scientific advances amidst many outstanding discoveries, to have to choose the two or three key investigators (who may not be the most visible or most cited ones), and to have to sift through many nominations that extol the achievements of some candidates (but overlook those of others). Nevertheless, the scientists selected in recent years have consistently been ones who have made outstanding contributions, and this year's decision to award the Nobel Prize for Chemistry to Avram Hershko, Aaron Ciechanover, and Irwin Rose for the discovery of the ubiquitin system is particularly insightful and honors three biochemists whose seminal work has altered modern biology.

For a long time, the physiological importance of intracellular protein breakdown was not widely recognized, and studies of this process were distinctly unfashionable. Today, undergraduates and even advanced high school students learn that the levels of proteins in cells are determined by the balance between their rates of synthesis and degradation, and that many cellular processes are regulated through the degradation of critical components by the ubiquitin-proteasome pathway. During the past 15 years, work of many investigators has firmly established the key role of this pathway in the control of cell growth, progression through the cell cycle (Peters, 2002; Deshaies, 1999; Hershko and Ciechanover, 1998), transcriptional regulation, apoptosis, immune responses (Ben-Neriah, 2002), and the pathogenesis of many human diseases (Glickman and Ciechanover, 2002; Sakamoto, 2002). For example, a number of cancers are associated with either a failure to degrade rapidly an oncoprotein or accelerated degradation of a tumor suppressor (Huibregtse et al., 1995; Guardavaccaro and Pagano, 2004; Kaelin, 2002), while apoptosis is inhibited under normal conditions by the selective destruction of various proapoptotic proteins (Martin, 2004). In fact, most changes in cell composition, whether major, as occurs during cell differentiation (Cook and Tyers, 2004), or limited, such as modifications of synaptic properties (Ehlers, 2003; Hegde and DiAntonio, 2002) or cell cycle transitions, involve the selective destruction of certain cell components and their replacement by others. Even the gross loss of neuronal integrity in Wallerian degeneration (Ehlers, 2004) and the marked loss of muscle mass resulting from inactivity, fasting, sepsis, and cancer cachexia results from a programmed activation of this degradative process (Lecker et al., 2004; Sandri et al., 2004).

*Correspondence: alfred_goldberg@hms.harvard.edu

In addition to its fundamental role in cell regulation, the ubiquitin-proteasome pathway serves as an essential quality control mechanism that selectively eliminates misfolded or damaged proteins from the cytosol and nucleus (Goldberg, 1972, 2003). A failure of this process leads to the accumulation of aberrant proteins within cells and appears to contribute to the pathogenesis of most of the major neurodegenerative diseases, including Parkinson's, Huntington's, Alzheimer's, and ALS (Sherman and Goldberg, 2001; Johnston and Madura, 2004). Eukaryotic cells also use the ubiquitin-proteasome pathway to rapidly destroy misfolded membrane proteins (e.g., Alzheimer's amyloid propeptide) before they emerge from the endoplasmic reticulum (Kostova and Wolf, 2003; Ahner and Brodsky, 2004). For example, in cystic fibrosis patients, the mutated membrane chloride transporter is recognized as misfolded, removed from the endoplasmic reticulum, and rapidly degraded. This degradative process also enables the immune system to selectively eliminate virus-infected or cancer cells, because peptides generated during the breakdown of cell proteins are continually displayed on MHC class I molecules on the surface of most cells (Rock and Goldberg, 1999). If foreign peptides are displayed, the cells are recognized by cytolytic T cells as potentially dangerous and are destroyed. Knowledge about this degradative process has already yielded practical benefits in medicine with the development of an important new anticancer therapy, the proteasome inhibitor Velcade (PS-341), which is already in wide use for the treatment of multiple myeloma and is presently in clinical trials against a number of other human cancers (Adams, 2004; Kisselev and Goldberg, 2001). It is in fact difficult to identify an area of biomedical research where protein degradation by the ubiquitin-proteasome pathway is not of major importance and a subject of active investigation.

The Ubiquitin-Proteasome Pathway

For these many reasons, it was widely anticipated that a Nobel Prize in Physiology and Medicine would someday be awarded in recognition of discoveries about this degradative process. In awarding the prize in Chemistry, however, the Nobel Committee chose to honor the seminal discoveries of the role of ubiquitin in this pathway and elucidation of the steps involved in its conjugation to proteins. This now classic work began when Avram Hershko was an Associate Professor of Biochemistry at the Technion in Haifa, Israel, and had as his PhD student Aaron Ciechanover. Together these MDs-turned biochemists discovered that the small heat-stable protein, later identified as ubiquitin, functions as a cofactor in the nonlysosomal pathway for protein breakdown (Ciechanover et al., 1978). Through a collaboration with Irwin Rose, an accomplished enzymologist at Fox Chase Cancer Institute in Philadelphia, they subsequently demonstrated that ubiquitin becomes covalently linked to

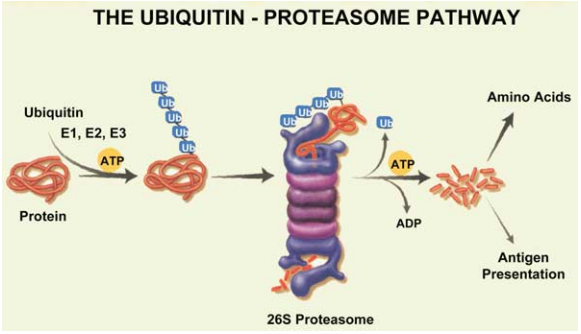

Figure 1. ATP Serves Multiple Functions in the Ubiquitin-Proteasome Pathway

proteins, and this modification targets them for rapid destruction (Figure 1).

The initial step in this process involves the ATP-dependent activation of ubiquitin to a thiol ester by the ubiquitin-activating enzyme E1 (Figure 2), which then transfers the highly reactive ubiquitin to one of the cell's 20 to 30 E2s or ubiquitin carrier proteins (Hershko and Ciechanover, 1998; Glickman and Ciechanover, 2002). The ubiquitin ligases or E3s bind specific protein substrates and thus confer substrate specificity to this process. The E3 then transfers the ubiquitin from the E2 onto an ϵ-amino group on a lysine on the protein substrate forming an unusual isopeptide bond in a T-shaped linkage (in contrast to the genetically encoded linear linkages of amino acids formed on ribosomes). The same E3 then links additional ubiquitin molecules to the preceding ubiquitin, and when the isopeptide chain contains more than four ubiquitin molecules (Thrower et al., 2000), the substrate is targeted to the 26S proteasome (Figure 3), where it is rapidly degraded to small peptides (Pickart and Cohen, 2004; Voges et al., 2000). Several different families of E3s have now been identified (Huibregtse et al., 1995; Jackson et al., 2000; Deshaies, 1999; Lorick et al., 1999; Weissman, 2001), and it is now recognized that mammalian cells contain between 500 and 1000 different ubiquitin ligases, which function with specific E2s to give exquisite selectivity to protein degradation, as well as to other ubiquitin-dependent processes (see below).

E3s generally recognize small domains of the substrate (often called "degrons") (Varshavsky, 1997). For example, a number of important E3s bind peptide sequences only when they are phosphorylated (Skowyra et al., 1997). This mechanism—phosphorylation by a specific kinase triggering selective ubiquitination and degradation of an enzyme or regulatory protein—underlies most of the irreversible steps in the cell cycle (Deshaies, 1999; Jackson et al., 2000), and also the onset of the inflammatory response, where TNF or other inflammatory mediators trigger the phosphorylation and rapid degradation of IκB, the inhibitor of NFκB transcription factor (Palombella et al., 1994; Ben-Neriah, 2002). A variety of cellular responses involve the selective degradation of a critical regulatory protein by a highly specific ubiquitin ligase, and the loss of a specific E3 can have consequences on brain function as divergent as mental retardation and Parkinson's disease (Johnston and Madura, 2004). One example of major importance in physiology and medicine is in the transcriptional adaptation of cells to anoxia (Kaelin, 2002; Krek, 2000). When cells are well oxygenated, a specific proline residue in the transcription factor HIF becomes oxidized to a hydroxyproline, and this minor modification is recognized by a specific E3. Consequently, in normal cells, the HIF is rapidly degraded and unable to function, but in anoxic cells, this modification can not occur; consequently, HIF is stable in ischemic cells and transcribes genes for erythroprotein, VEGF, and glycolytic enzymes. This adaptation is especially important for the growth of cancer cells, some of which (e.g., renal cell carcinomas, pheochromocytomas) lose this specific E3 (Kaelin, 2002), which thus functions as a tumor suppressor.

These various advances have resulted from the work of many talented biochemists and cell biologists. However, our present understanding of the ubiquitin-proteasome pathway stems from the initial elucidation by Hershko, Ciechanover, and Rose of the biochemical reactions involved in protein ubiquitination, as well as the elegant genetic analysis of this process and its components by Varshavsky and coworkers (Varshavsky, 1997; Finley et al., 1984), which stimulated much wider interest in this area. The subsequent discovery of the 26S proteasome (Figure 3) (Hough et al., 1987; Waxman et al., 1987) and the recent identification of many new functions of this pathway, largely through the use of the pharmacological inhibitors of the proteasome (Rock et al., 1994; Kisselev and Goldberg, 2001), have led to our present understanding of this pathway and its biological importance.

Discovery of the Nonlysosomal Pathway and of Ubiquitin Conjugation

The work of Hershko and colleagues initially focused on trying to understand an anomalous observation that, if real, appeared to be an important clue to the identification of the site and biochemical mechanisms for intracellular protein degradation. Several investigators had observed that inhibitors of ATP production could block protein breakdown and suggested that this process required ATP (Goldberg and St. John, 1976; Ciechanover, 2004), which was a very surprising suggestion, because protein hydrolysis is a thermodynamically favored reaction, and all proteases known then (e.g., digestive or lysosomal proteases) functioned independently of ATP. In fact, these reports actually were not taken seriously by most biochemists at the time, since they involved use of metabolic poisons and could well have been explained by various indirect effects (e.g., ATP might be required for synthesis of new proteases or to maintain the redox state or ionic milieu in the cells). Alternatively, these observations could indicate that ATP was required for the function of lysosomes or to transport proteins into this organelle, which was generally assumed to be the site for intracellular proteolysis (de Duve and Wattiaux, 1966).

Consequently, in the early 1970s, the present author spent several years critically analyzing this requirement in intact cells, only to finally establish that ATP was truly

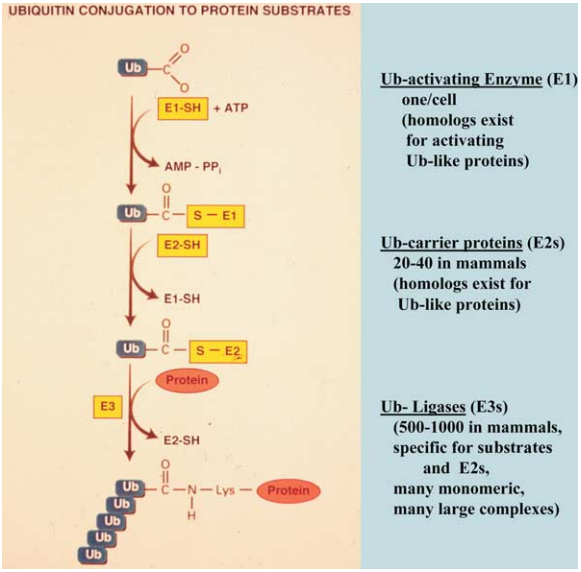

Figure 2. The Three Steps in the Formation of a Ubiquitin Chain on a Protein Substrate

required for an early step in the breakdown process (Goldberg et al., 1975; Goldberg and St. John, 1976). We had chosen to study this process in reticulocytes and *E. coli*, specifically because they lack lysosomes, and using extracts of these cells, we eventually were able to demonstrate the existence of a nonlysosomal proteolytic system that utilized ATP to catalyze the rapid degradation of misfolded cell proteins (Etlinger and Goldberg, 1977). The roles of ATP and the responsible degradative machinery were then amenable to biochemical analysis. Using such preparations from reticulocytes, Hershko, Ciechanover, and Rose discovered the involvement of ubiquitin and the critical role of ATP hydrolysis in providing the energy necessary for covalent linkage of ubiquitin to the substrate. (For a fuller account of these early developments, see the press release of the Royal Swedish Academy of Sciences [Nobelprize.org] or the recent review by Ciechanover [2004]).

Since my lab had laid the basis for their seminal work, but missed the discovery of ubiquitin, I am in a unique position to appreciate the truly innovative character of their discoveries, and how it provided a new conceptual framework for the studies of protein turnover, and especially the "selectivity problem." Since the initial recognition by Schimke and coworkers in the 1960s that different proteins in cells are degraded at distinct rates and that rapid degradation is a key feature of highly regulated enzymes (Schimke and Doyle, 1970), the biochemical basis of this selectivity and for the selective degradation of misfolded proteins (Goldberg, 1972) was an active topic of speculation amongst the few individuals investigating this area. This rapid degradation of rate-limiting enzymes and misfolded proteins indicated that protein half-lives must be determined by their tertiary or primary sequences (Goldberg and Dice, 1974) and seemed inconsistent with degradation within lysosomes. It is now clear that this selectivity reflects the existence in cells of very many highly specific E3s.

However, the importance of ubiquitin was not immediately apparent after the initial reports from Hershko and Ciechanover; in fact, at the time, we (and others) were initially atheistic concerning the "ubiquitin hypothesis," but then we became agnostic, and soon we were firm believers. Our conversion came about due to their subsequent series of elegant papers, many with I. Rose, that appeared in the early 1980s and defined the enzymatic steps in the ubiquitination process (Hershko et al., 1980, 1981, 1983, 1984; Ciechanover et al., 1978, 1980, 1981). Amongst biologists, the contributions of Hershko and Ciechanover are widely recognized because of their major roles in the subsequent development of this area and their useful reviews. Rose's contributions have been less widely appreciated, but elucidation of the ubiquitination pathway required a sophistication in enzymology that few biologists possess or appreciate, such as their use of a pyrophosphate exchange assay for the enzyme E1, defining the key roles of thiol esters in ubiquitin activation (enzymatic reactions resembling those involved in fatty acid oxidation), their discovery of the isopeptidases that disassemble ubiquitin chains, or the synthesis of ubiquitin-aldehyde to inhibit this process, and biochemical mechanisms. These major contributions were certainly made possible by Rose's enzymological expertise, and it is a sign of Hershko's cleverness to seek out such a knowledgeable collaborator.

However, there was also an important element of serendipity in this story. When the Israeli workers came to work with Rose at the Fox Chase Institute in Philadelphia, a neighboring lab was studying the function of histone A24, an unusual histone, which Harris Busch and colleagues had shown consists of a histone linked to the polypeptide ubiquitin through an isopeptide linkage (Goldknopf and Busch, 1977). Familiarity with the work of their neighbors enabled Wilkinson and Haas (two postdocs in Rose's lab) to show that ubiquitin corresponded to the heat-stable polypeptide that Hershko and Ciechanover had implicated in proteolysis (Wilkinson et al., 1980), and this recognition led to a unifying hypothesis by the three prize winners that ubiquitin ligation to proteins targets them for destruction (Hershko et al., 1980), as they subsequently demonstrated.

The 26S Proteasome and a Fuller Understanding of the ATP Requirement for Proteolysis

Ironically, it soon became clear that ubiquitin conjugation does not explain the ATP requirement for intracellular proteolysis, and further exploration of this requirement led to a much fuller understanding of this pathway. Ubiquitin first appeared during evolution with the appearance of eukaryotes, presumably to provide greater selectivity and regulation to the degradative process. In prokaryotes and their descendants, mitochondria and chloroplasts (Desautels and Goldberg, 1982), protein breakdown shows a similar ATP dependence (Goldberg and St. John, 1976), although they lack ubiquitin. In fact, for this reason, we were initially slow to believe in ubiquitin conjugation as the explanation of the ATP requirement, especially since in 1980 we discovered

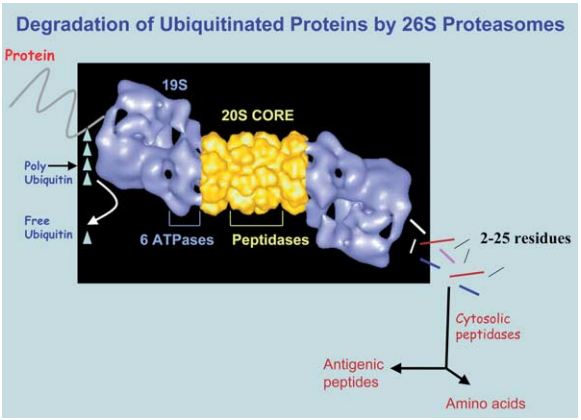

Figure 3. The 26S Proteasome Binds the Ubiquitin Chain and Uses ATP to Unfold and Translocate the Substrate into the 20S Core Particle for Degradation

This figure was modified from an electron microscopic tomograph of the 26S proteasome kindly provided by W. Baumeister.

that, in bacteria, the energy requirement for selective proteolysis was due to the involvement of a new kind of proteolytic enzyme that digests proteins and ATP in linked reactions without any covalent tagging of the substrate (Chung and Goldberg, 1981; Chung, 1993; Gottesman, 1996). Such ATP-hydrolyzing proteases are multisubunit complexes, many times larger than typical proteases, and prokaryotes and mitochondria contain several such enzymes. Thus, two opposite explanations for the ATP requirement for intracellular proteolysis emerged at about the same time, and subsequent work established that both mechanisms function in the ubiquitin-dependent pathway.

By the mid-1980s, it became clear even that after proteins are ubiquitinated, ATP is still necessary for their breakdown (Tanaka et al., 1983; Hershko et al., 1984), and several years later, the 26S proteasome (Figure 3), the very large ATP-dependent proteolytic complex that degrades ubiquitinated proteins, was identified (Hough et al., 1987; Waxman et al., 1987). This huge molecular machine functions in analogous ways to the ATP-dependent proteases and proteasomes in prokaryotes (Pickart and Cohen, 2004). They utilize the energy in ATP to unfold globular proteins and to translocate them into an internal proteolytic chamber (i.e., the 20S core proteasome) in which protein hydrolysis occurs in an isolated environment away from valuable cellular components (Pickart and Cohen, 2004; Benaroudj et al., 2003; Voges et al., 2000).

More recent work has indicated that ATP is also necessary for additional steps that are critical in the recognition of substrates for ubiquitination. As noted above, many regulatory proteins are marked for ubiquitination by specific kinases, which means that their breakdown requires ATP in the very first step. In addition, the selective degradation of mutant or misfolded proteins in the cytosol or ER, including various proteins important in human diseases (CFTR, hyperphosphorylated tau), occurs through a collaboration between ubiquitin ligases and the molecular chaperones, especially Hsp70 and 90, which selectively bind the abnormal species and in the presence of ATP release them for ubiquitination by the specific E3, e.g., CHIP (Cyr et al., 2002; Murata et al., 2001). Also, eukaryotic cells utilize a specific ATPase complex (p97) to extract misfolded secretory proteins from the ER for ubiquitination and degradation in the cytosol (Kostova and Wolf, 2003; Ahner and Brodsky, 2004). Finally, ATP depletion of cells (as done in the early experiments of Hershko and Ciechanover) causes disassembly of the 26S proteasome and of its 19S regulatory complex, and one function of ATP in the extracts (and perhaps also in vivo) is to support the reassembly of the 26S proteasome (Imai et al., 2003; Eytan et al., 1989; Driscoll and Goldberg, 1990). In any case, it is noteworthy that attempts to understand a biochemical anomaly—why a thermodynamically favored process should require metabolic energy—has led to the discovery of novel biochemical machinery and a wealth of new biological insights.

Other Functions of Ubiquitin and the Ubiquitin-like Proteins

More recently, the postsynthetic modification of proteins by linkage to ubiquitin has been discovered to function as a key marking step in other cellular processes not involving the proteasome (Pickart, 2001). In fact, surprisingly, ubiquitin and ubiquitin-like proteins have recently been shown to play a key role in the incorporation of proteins into the lysosome, both endocytosed proteins and cytosolic proteins engulfed in autophagic vacuoles. Ligation of a single ubiquitin to membrane receptors or transport proteins leads to their endocytosis and targets them for degradation by lysosomes (Hicke, 2001; Marmor and Yarden, 2004; Holler and Dikic, 2004). The formation on an intracellular protein of a different type of ubiquitin chain than causes proteasomal degradation (i.e., ones involving isopeptide linkages to different lysines on the ubiquitin) are key events in the repair of damaged DNA and in certain signal transduction cascades, including the induction of the inflammatory response by TNFa (Deng et al., 2000). In addition, eukaryotes have evolved a number of ubiquitin homologs, other small proteins (with arcane names like Sumo, Nedd8, or ISI15) that also get ligated to specific protein substrates through similar reactions. These modifications serve important physiological roles that are different from those of ubiquitin conjugation (Schwartz and Hochstrasser, 2003; Johnson, 2004; Mizushima et al., 2003); for example, conjugation to Sumo targets proteins to the nuclear envelope and nuclear pore complex, and conjugation of NedD8 to multisubunit ubiquitin ligases (e.g., SCF complexes) causes their activation, while ligation of the atg proteins, of atg8 to a membrane protein and atg12 to a membrane lipid, triggers the formation of autophagic vacuoles during cell starvation and apoptosis (Ohsumi and Mizushima, 2004). The conjugation of each ubiquitin-like protein involves activation by a specific E1, its transfer to a specific E2, and covalent ligation to lysine residues on the regulated proteins. Thus, the biochemistry of ubiquitination, recognized by this award, actually plays more ubiquitous roles in the functioning of eukaryotic cells than ubiquitin itself.

An Afterthought

This Nobel Prize is unusual in an additional respect. This prize represents the first awarded to Israeli scientists for work in Israel, and it is indeed rare, perhaps unprecedented, for outstanding research of an experimental nature to originate in a small country with limited resources, especially one where frequent wars and military challenges make enormous demands on its resources and its citizens. Nevertheless, important contributions to our knowledge about protein breakdown and to other areas of cell biology continue to emerge from Israeli laboratories to the benefit of biomedical science worldwide.

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