VIEWPOINT

Discovery of the Biology of the Ubiquitin System

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The 2014 Albany Medical Center Prize in Medicine and Biomedical Research has been awarded to Dr Alexander Varshavsky in recognition of major scientific contributions to the biological sciences. This Viewpoint provides a summary of those discoveries, including the history of early research on the ubiquitin system and intracellular protein degradation, and discusses the implications of these scientific advances for medicine.

This article, about the discovery of the biology of the ubiquitin system, describes my laboratory's main contribution to date, and stems, in part, from previous historical summaries.[1-3] Another antecedent is the interview given in 2006 to Dr Istvan Hargittai, a distinguished Hungarian chemist. It described my life and science, including the early years in Moscow (Russia), the 1977 emigration from the former Soviet Union to the United States, and scientific work that ensued.[4]

Protein Degradation and the Ubiquitin System

Proteolysis (protein degradation) is mediated by proteases, which range from relatively small monomeric proteins to large multisubunit proteases called proteasomes. For a very long time, and despite some evidence to the contrary, most intracellular proteins were thought to be long-lived. This assumption survived nearly intact until the 1980s, when 2 complementary sets of discoveries were made, largely by 2 groups, the laboratory of Avram Hershko at the Technion (Haifa, Israel) and my laboratory, then at Massachusetts Institute of Technology (Cambridge). Through the elegant use of biochemical fractionation and enzymology, Hershko and coworkers discovered in 1978-1980 that some proteins added to a mammalian cell extract became covalently conjugated to a small (76-residue) protein called ubiquitin, and that ubiquitylated proteins were destroyed by an adenosine triphosphate (ATP)-dependent protease in the cell extract.[1,5] (This protease was characterized by several laboratories much later, in the 1990s, and is now called the 26S proteasome.[6]) In 1981-1983, Hershko and coworkers identified a set of enzymes termed E1, E2, and E3 that mediate the conjugation of ubiquitin and thereby confer short half-lives on proteins that become ubiquitylated.[1]

In 1984-1990, these mechanistic advances with cell-free systems and isolated enzymes were complemented by our laboratory's function-based discoveries with mammalian cells and the yeast *Saccharomyces cerevisiae*.[2,3] These insights revealed the singularly important biology of the ubiquitin system, including the first demonstration that the bulk of protein degradation in living cells requires ubiquitin conjugation, and the identification of the first ubiquitin-conjugating (E2) enzymes with specific physiological functions, in the cell cycle (the Cdc34 E2 enzyme) and DNA repair (the Rad6 E2 enzyme).[2,3] These advances initiated the understanding of the massive, multilevel involvement of the ubiquitin system in the regulation of the cell cycle progression and DNA damage responses. Specifically, many regulators of the cell division cycle are conditionally short-lived proteins that are destroyed in precisely controlled spatiotemporal patterns. Adaptive reactions of cells to DNA damage require the ubiquitin system both for the regulated degradation of some DNA repair proteins and for modifying, though ubiquitylation, specific properties of other proteins that control DNA repair. We also discovered critical functions of the ubiquitin system in stress resistance, protein synthesis, and transcriptional regulation.[2,3]

In 1990, we identified and cloned an E3 enzyme termed Ubr1, the first molecularly cloned and analyzed E3 ubiquitin ligase.[3] Together with the Rad6 and Cdc34 results, the cloning and analysis of the Ubr1 E3 created a particularly large arena of research, as it became known, through studies by many laboratories, that the mammalian genome encodes at least 1000 distinct E3s. The targeting of many (most) cellular proteins by this remarkable variety of specific E3 ubiquitin ligases underlies the immense functional reach of the ubiquitin system.

Other key discoveries by our laboratory in the 1980s included (1) the necessity of polyubiquitylation, in which ubiquitin moieties are covalently joined to each other, forming a specific polyubiquitin chain that is linked to a substrate protein and is required for its degradation; (2) the subunit selectivity of protein degradation (ie, the ability of the ubiquitin system to destroy a specific subunit of a multisubunit complex while leaving the rest of the complex intact); (3) MATα2, a transcriptional repressor, as the first physiological substrate of the ubiquitin system (before this advance, the ubiquitin system was examined using artificial substrates); and (4) the first genes that encode ubiquitin precursor proteins and deubiquitylating enzymes.[2,3]

Degradation Signals and the N-End Rule Pathway

Since the beginning of ubiquitin studies, it was surmised that short-lived proteins contained specific degradation signals that mediate ubiquitylation and degradation of proteins by the ubiquitin system, but the nature of those signals was a mystery. In 1986, we discovered the first degradation signals (degrons) in short-lived proteins.[3,7] These signals included degrons that give rise to the N-end rule, which relates the in vivo half-life of a protein to the identity of its N-terminal residue. The N-end rule pathway recognizes proteins containing N-terminal degradation signals called N-degrons, polyubiquitylates these proteins, and thereby causes their degradation by the proteasome.[8]

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Over the last 2 decades, the regulated degradation of proteins or their fragments by the N-end rule pathway has been shown, by us and by others, to mediate a broad range of biological functions, including the sensing of heme, nitric oxide, oxygen, and short peptides (through the ability of these small compounds to modulate the degradation of specific N-end rule substrates); the control of stoichiometries of subunits in oligomeric protein complexes (through the degradation, by the N-end rule pathway, of newly formed proteins until they become protected parts of multisubunit complexes); the elimination of misfolded proteins; the repression of apoptosis and neurodegeneration; the regulation of chromosome transcription, repair, replication, and cohesion/segregation; the regulation of G proteins, autophagy, peptide import, meiosis, immunity, fat metabolism, cell migration, actin filaments, cardiovascular development, spermatogenesis, neurogenesis, and long-term memory in animals; and the regulation of many processes in plants.[8-10]

Biological Functions of the Ubiquitin System

By the end of the 1980s, our studies had revealed the major biological functions of the ubiquitin system as well as the basis for its specificity (ie, the first degradation signals in short-lived proteins). The resulting discovery of physiological regulation by intracellular protein degradation has transformed the understanding of biological circuits, as it became clear that control through regulated protein degradation rivals, and often surpasses in significance the classical regulation through transcription and translation.[3,8] In other words, it has become clear that the regulation of in vivo levels (concentrations) of many specific proteins was determined not only by the rates of their synthesis (through transcription and translation) but also—and often largely—by the rates of selective degradation of these proteins. Just how extensive and elaborate ubiquitin functions are was understood more systematically and in great detail over the next 2 decades, through studies by many laboratories that began entering this field in the 1990s, an expansion that continues to the present day.

One of new directions in later studies by other laboratories involved a family of ubiquitin-like proteins (UBLs), which can be ubiquitin-like not only in their structural similarity to ubiquitin, but also in their ability to be conjugated to other proteins. The functions of UBLs encompass a great variety of processes, including the autophagy (in which proteins are sequestered in membrane-enclosed vesicles and thereafter destroyed by proteases inside the organelles called lysosomes), the nuclear transport of specific proteins, the cohesion/segregation of replicated chromosomes, the repair of DNA, and a multitude of signal transduction pathways.

Ubiquitin and Medicine

The ubiquitin and ubiquitin-like systems are of major importance in medicine, given their immense functional range and many ways in which these circuits can malfunction in disease, from cancer and neurodegenerative syndromes to perturbations of immunity and other illnesses, including birth defects. Most of these malfunctions are caused by mutations in distinct genes that either repress or overinduce the expression of specific components of the ubiquitin system, or alter (eg, inactivate) these components, or change specific substrates of the ubiquitin system, making them, for example, longer-lived in vivo. Parkinson disease, specific cancers resulting from oncogenic viral infections or from mutations in proteins that mediate DNA repair, and birth defects that involve, in particular, mental retardation and are caused by inactivation of specific ubiquitin ligases (eg, the Angelman syndrome and the Johanson-Blizzard syndrome) are just a few of many diseases that are known to be caused, in whole or in part, by perturbations of the ubiquitin and ubiquitin-like systems.

Both academic laboratories and pharmaceutical companies are developing compounds that target specific components of these systems. The fruits of their labors have already become, or will soon become, clinically useful drugs. Work in this arena may produce not only "conventional" inhibitors or activators of specific enzymes, but also drugs that would direct the ubiquitin system to target, destroy, and thereby down-regulate any specific protein.

After 3 decades of ever-expanding studies in this vast biomedical realm, new directions of inquiry, new problems, and new applications of fundamental discoveries continue to accumulate at a clip that exceeds anyone's ability to follow these studies in their entirety, a state of affairs that is frustrating and exhilarating at the same time. Major, unexpected breakthroughs in this arena are still likely to occur and would be accompanied by further advances in the application of fundamental understanding to problems in clinical medicine. I feel privileged having been able to contribute to the birth of this field, and to partake in its later development.

ARTICLE INFORMATION

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REFERENCES

1. Hershko A, Ciechanover A, Varshavsky A. Basic Medical Research Award: the ubiquitin system. *Nat Med*. 2000;6(10):1073-1081.

2. Varshavsky A. The early history of the ubiquitin field. *Protein Sci*. 2006;15(3):647-654.

3. Varshavsky A. Discovery of cellular regulation by protein degradation. *J Biol Chem*. 2008;283(50):34469-34489.

4. Varshavsky A. In: Hargittai I, Hargittai M, eds. *Candid Science.* Vol 6. London, UK: Imperial College Press; 2006:311-359.

5. Hershko A, Ciechanover A, Heller H, Haas AL, Rose IA. Proposed role of ATP in protein breakdown. *Proc Natl Acad Sci U S A*. 1980;77(4):1783-1786.

6. Förster F, Unverdorben P, Sledź P, Baumeister W. Unveiling the long-held secrets of the 26S proteasome. *Structure*. 2013;21(9):1551-1562.

7. Bachmair A, Finley D, Varshavsky A. In vivo half-life of a protein is a function of its amino-terminal residue. *Science*. 1986;234(4773):179-186.

8. Varshavsky A. The N-end rule pathway and regulation by proteolysis. *Protein Sci*. 2011;20:1298-1345.

9. Shemorry A, Hwang C-S, Varshavsky A. Control of protein quality and stoichiometries by N-terminal acetylation and the N-end rule pathway. *Mol Cell*. 2013;50(4):540-551.

10. Kim H-K, Kim RR, Oh JH, Cho H, Varshavsky A, Hwang CS. The N-terminal methionine of cellular proteins as a degradation signal. *Cell*. 2014;156(1-2):158-169.