# Preface

## Ubiquitin Wins Nobel

Eyal Reinstein MD PhD

Department of Medicine B, Sheba Medical Center, Tel Hashomer, Israel

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The 2004 Nobel Prize in Chemistry was awarded to three scientists – Avram Hershko, Aaron Ciechanover (both from the Technion-Israel Institute of Technology) and Irwin Rose (from the University of California at Irvine, United States) – for the discovery of the ubiquitin-mediated protein degradation. Since the ubiquitin system controls key metabolic pathways, the combined medical-biochemical research on the system holds great promise for the future understanding and treatment of various diseases. A special section in this issue of the *Israel Medical Association Journal* is dedicated to the ubiquitin system and to this prestigious achievement of Israeli scientists. Last year, the Israel Academy of Sciences and Humanities held a symposium in honor of the Nobel Laureates. The papers in this issue are a collection of the presentations given on that occasion.

How did it all begin? In the late 1970s, Hershko and Ciechanover, both physicians and biochemists, first reported that a small, heat-stable protein that they named APF-1 (for ATP-Dependent Proteolytic Factor 1) is an essential component of an energy-dependent proteolytic system in rabbit reticulocyte extracts. Their paper, entitled "A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes," was published in *Biochemical and Biophysical Research Communications* [1], a second-tier journal; nevertheless, it paved the way for subsequent groundbreaking detailed studies by this group and others. APF-1 was later shown to be identical to a 76 amino acid protein named ubiquitin for its ubiquitous expression in all cell types. Hershko and Ciechanover carried out an essential part of the work leading to the Nobel Prize in the laboratory of Irwin Rose, then at the Fox Chase Cancer Center in Philadelphia. The three scientists demonstrated that ubiquitin, whose function was unknown at the time, is covalently linked to protein substrates and that it is necessary for the rapid degradation of these proteins in their reticulocyte system. It was only a few years later, in studies that demonstrated the physiologic impact of protein degradation mediated by the ubiquitin system, that the importance of their initial findings was fully appreciated.

What is the biological significance of the ubiquitin system? The ubiquitin system is regarded as the major pathway for regu-

INTRACELLULAR PROTEIN DEGRADATION: FROM A VAGUE IDEA THRU THE LYSOSOME AND THE UBIQUITIN-PROTEASOME SYSTEM AND ONTO HUMAN DISEASES AND DRUG TARGETING

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by

AARON CIECHANOVER

Cancer and Vascular Biology Research Center, Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel.

ABSTRACT

Between the 1950s and 1980s, scientists were focusing mostly on how the genetic code is transcribed to RNA and translated to proteins, but how proteins are degraded has remained a neglected research area. With the discovery of the lysosome by Christian de Duve it was assumed that cellular proteins are degraded within this organelle. Yet, several independent lines of experimental evidence strongly suggested that intracellular proteolysis is largely non-lysosomal, but the mechanisms involved had remained obscure. The discovery of the ubiquitin-proteasome system resolved this enigma. We now recognize that ubiquitin- and proteasome-mediated degradation of intracellular proteins is involved in regulation of a broad array of cellular processes, such as cell cycle and division, regulation of transcription factors, and assurance of the cellular quality control. Not surprisingly, aberrations in the system have been implicated in the pathogenesis of many human diseases, malignancies and neurodegenerative disorders among them, which led subsequently to an increasing effort to develop mechanism-based drugs, one is already in use.



Eyal Reinstein completed the MD/PhD program in 2004 at the Technion-Israel Institute of Technology, Haifa, under the supervision of Prof. Aaron Ciechanover.

APF-1 = ATP-dependent proteolytic factor 1

lated degradation of intracellular proteins. For the past few years, it is almost impossible to conduct research in the biomedical sciences without coming across the ubiquitin system. Intracellular protein degradation plays many important roles. One major function is to maintain the cell's quality control by removing mutated or damaged proteins. Another important function is to control basic cellular processes, including distinct metabolic pathways, cell cycle, and transcription, via the removal of key regulatory proteins. Not surprisingly, aberrations in the ubiquitin system have been implicated in the pathogenesis of numerous human diseases, including malignancies, neurodegenerative diseases and muscle wasting. Consequently, pharmacologic manipulation of the ubiquitin system has the potential to alter the outcome of many diseases, especially malignancies. Bortezomib is the first drug on the market that inhibits ubiquitin system activity; it is indicated for the treatment of multiple myeloma.

The ubiquitin pathway for degradation of intracellular proteins is conceptually and mechanistically different from the degradation of dietary proteins. While the degradation of dietary proteins in the gastrointestinal tract is mediated by non-specific proteases such as pepsin, trypsin and chymotrypsin, and thermodynamically releases energy, intracellular degradation is specific and selective and it consumes rather than produces metabolic energy. It should be noted that ubiquitin-mediated intracellular proteolysis is also different from the non-specific lysosomal degradation of extracellular proteins such as serum proteins, lipoproteins, and peptide hormones. These proteins are digested at a similar rate after being endocytosed from the blood into the cell.

How does the cell select, in a highly specific manner, proteins that are destined for degradation? It does so by the attachment of ubiquitin chains to these proteins, tagging that marks them for destruction (see journal cover and legend). The studies of the three scientists established the sequence of reactions that leads to protein ubiquitination and degradation: a) Conjugation of multiple ubiquitin molecules to the target protein, a process that is catalyzed by three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and a substrate-specific E3 (ubiquitin-protein ligase). b) Degradation of the tagged substrate by the 26S proteasome complex that recognizes only ubiquitin-conjugated proteins and degrades them to small peptides, releasing free and reusable ubiquitin.

The collection of papers presented here begins with the abstract of Aaron Ciechanover's Nobel lecture. This paper describes the chain of events that led from the idea of ATP-dependent protein degradation, to the selection of the experimental system and the technological challenges and finally to the description of a novel biochemical pathway for protein degradation. Daniel Kornitzer highlights the involvement of the ubiquitin system in the morphogenesis of the pathogenic fungus *Candida albicans* from yeast form to the hyphal form, a process that is related to its virulence. Mono-ubiquitination (the attachment of a single ubiquitin molecule to the substrate), until recently an elusive phenomenon, has emerged as a reversible modification that controls the trafficking of membrane proteins between various cellular compartments. Yaron Mosesson and Yosef Yarden review the role of this novel non-proteolytic modification in protein transport. Shoshana Bar-Nun and colleagues, studying the ERAD (Endoplasmic Reticulum Associated Degradation) quality control mechanism that retains misfolded, damaged and mutated proteins for degradation by the ubiquitin system, discuss the signals in the immunoglobulin M heavy chain that confer retention and degradation of the protein. The tumor suppressor protein p53 is a well-studied substrate of the ubiquitin system. One of the main roles of p53 is to respond to DNA damage caused by genotoxic agents, by halting cell division and inducing repair mechanisms. If the damage is irreparable, p53 induces apoptosis. Gad Asher and Yosef Shaul demonstrate an alternative pathway for p53 degradation – ubiquitin-independent but proteasome-dependent – suggesting that p53 is one of several inherently unstable proteins. The arf tumor suppressor protein activates p53 by antagonizing its ubiquitin ligase. Arf itself is degraded by the ubiquitin system via a recently identified distinct mechanism in which the ubiquitin molecules are attached to its N-terminal amino acid rather than to internal lysine residue. Willem den Besten and colleagues describe the regulatory mechanisms that control Arf expression and activities.

Despite the large body of accumulated knowledge generated on the mechanisms of action and pathophysiology of ubiquitination, the unknown far exceeds what we currently know of the ubiquitin system. What we can certainly anticipate in the near future is the continuous identification of substrates and their specific ubiquitin enzymes. Hopefully, this will unravel some of the mysteries surrounding the mechanisms of specific recognition of the substrates of the system and the involvement of the ubiquitin system in human pathologies.

## References

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**Correspondence:** Dr. E. Reinstein, Dept. of Medicine B, Sheba Medical Center, Tel Hashomer 52621, Israel.
Phone: (972-3) 530-2652
Fax: (972-3) 535-2855
email: eyalr@mit.edu

*We will bankrupt ourselves in the vain search for absolute security*

Dwight David Eisenhower (1890-1969), U.S. general and 34th president