Our main subject is the ubiquitin-proteasome system. The field of ubiquitin and regulated protein degradation was created in the 1980s, largely through the complementary discoveries by the laboratory of A. Hershko (Technion, Israel) and by my laboratory, then at MIT. The important mechanistic discovery, in 1978-1985, by Hershko and coworkers revealed ubiquitin-mediated proteolysis and E1-E3 enzymes of ubiquitin conjugation in vitro (in cell-free settings), while the complementary studies by our laboratory, in 1982-1990, discovered biological (in vivo) fundamentals of the ubiquitin system.
Our contributions in the 1980s comprised the discovery of a major role of ubiquitin conjugation in the bulk protein degradation in living cells; the discovery of the first degradation signals (termed degrons) in short-lived proteins and the multi-determinant nature of these signals; the first specific pathways of the ubiquitin system (the N-end rule pathway and the ubiquitin-fusion-degradation (UFD) pathway); the subunit selectivity of protein degradation (a fundamental capability of the ubiquitin system); the first non-proteolytic function of ubiquitin (its role as a chaperone in the biogenesis of ribosomes); and the first specific biological functions of the ubiquitin system, including its major roles in the cell cycle progression, in stress responses, in protein synthesis, in DNA repair, in chromosome cohesion/segregation, and in transcriptional regulation. These advances included the discovery of the first ubiquitin-conjugating (E2) enzymes with specific physiological functions, in the cell cycle (CDC34) and DNA repair (RAD6). These insights initiated the understanding of the massive, multilevel involvement of the ubiquitin system in the regulation of the cell cycle and DNA damage responses.

We also discovered the first specific (Lys48-type) substrate-linked polyubiquitin chains and their necessity for proteolysis; the first genes encoding ubiquitin precursors (linear polyubiquitin and ubiquitin fusions to specific ribosomal proteins); the MATα2 repressor as the first physiological substrate of the ubiquitin system; and the first specific E3 ubiquitin ligase, UBR1, which was identified, cloned and analyzed in 1990. The latter advance opened a particularly large field, because we the mammalian genome turned out to encode more than 1,000 distinct E3s. The targeting of many distinct degrons in cellular proteins by this immense diversity of E3 ubiquitin ligases underlies the unprecedented functional reach of the ubiquitin system.

Other contributions by our laboratory include the discovery of the first nucleosome-depleted (nuclease-hypersensitive) sites in chromosomes (in 1978-79), and the first chromosome cohesion/segregation pathway, via the topoisomerase 2-mediated decatenation of multicatenated (multiply intertwined) sister chromatids (in 1980-81).

We also developed new methods in biochemistry and genetics, including the ubiquitin fusion technique (in 1986); the chromatin immunoprecipitation assay (ChIP, in 1988; it was called ChIP by later users of this technique); a temperature-sensitive (ts) degron as a new way to make ts mutants (in 1994); the split-ubiquitin assay for in vivo protein interactions (in 1994); the ubiquitin sandwich assay for detecting and measuring cotranslational proteolysis (in 2000); and other new methods as well.

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, i.e., the first degradation signals in short-lived proteins. The resulting discovery of the 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. Just how strikingly broad and elaborate ubiquitin functions are was understood more systematically and in great detail over the next two decades, through studies by many laboratories that began entering this field in the 1990s, an expansion that continues to the present day. For accounts of the early history of the ubiquitin field, see Hershko et al. (2000); Varshavsky (2006, 2008, 2012, 2014).
Recent Research

Our current work at Caltech continues to focus on the ubiquitin system, with an emphasis on the N-end rule pathway. This pathway recognizes proteins containing N-terminal degradation signals called N-degrons, polyubiquitylates these proteins and thereby causes their degradation by the proteasome. The main determinant of an N-degron is a destabilizing N-terminal residue of a protein. Recognition components of the N-end rule pathway are called N-recognins. In eukaryotes, N-recognins are E3 ubiquitin ligases that can target N-degrons. Bacteria also contain a version of the N-end rule pathway.

Regulated degradation of proteins or their fragments by the N-end rule pathway mediates a strikingly broad range of functions, including the sensing of heme, nitric oxide, oxygen, and short peptides; control of protein quality and subunit stoichiometries, including the elimination of misfolded proteins; regulation of signaling by G proteins; repression of neurodegeneration; regulation of apoptosis, chromosome cohesion/segregation, transcription, and DNA repair; control of peptide import; and regulation of meiosis, autophagy, immunity, fat metabolism, cell migration, actin filaments, cardiovascular development, spermatogenesis, and neurogenesis; the functioning of adult organs, including the brain, muscle and pancreas; and the regulation of many processes in plants.

In eukaryotes, the N-end rule pathway consists of two branches. One branch, discovered in 1986, is called the Arg/N-end rule pathway. It targets specific unacetylated N-terminal residues. The “primary” destabilizing N-terminal Arg, Lys, His, Leu, Phe, Tyr, Trp, and Ile are directly recognized by N-recognins. The unacetylated N-terminal Met, if it is followed by a bulky hydrophobic (∅) residue, also acts as a primary destabilizing residue (Kim et al., 2014). In contrast, unacetylated N-terminal Asn, Gln, Asp, and Glu (as well as Cys, under some metabolic conditions) are destabilizing owing to their preliminary modifications, which include N-terminal deamidation (Nt-deamidation) of Asn and Gln and Nt-arginylation of Asp, Glu and oxidized Cys (Piatkov et al., 2012, 2014; Brower et al., 2013; Varshavsky, 2011).

The pathway’s other branch, discovered and characterized quite recently (in 2010-2014), is called the Ac/N-end rule pathway. It targets proteins for degradation through their Nα-terminally acetylated (Nt-acetylated) residues. Degradation signals and E3 Ub ligases of the Ac/N-end rule pathway are called Ac/N-degrons and Ac/N-recognins, respectively. Nt-acetylation of cellular proteins is apparently irreversible, in contrast to acetylation-deacetylation of internal Lys residues. Approximately 90% of human proteins are cotranslationally Nt-acetylated by ribosome-associated Nt-acetylases. Many, possibly most, Nt-acetylated proteins contain Ac/N-degrons pathway (Hwang et al., 2010; Shemorry et al., 2013; Kim et al., 2014).

References cited:


For more information, please click [here](#).

**PUBLICATIONS**

**2014**


2013

