PERSPECTIVE

N-degron and C-degron pathways of protein degradation

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This perspective is partly review and partly proposal. N-degrons and C-degrons are degradation signals whose main determinants are, respectively, the N-terminal and C-terminal residues of cellular proteins. N-degrons and C-degrons include, to varying extents, adjoining sequence motifs, and also internal lysine residues that function as polyubiquitylation sites. Discovered in 1986, N-degrons were the first degradation signals in short-lived proteins. A particularly large set of C-degrons was discovered in 2018. We describe multifunctional proteolytic systems that target N-degrons and C-degrons. We also propose to denote these systems as "N-degron pathways" and "C-degron pathways." The former notation replaces the earlier name "N-end rule pathways." The term "N-end rule" was introduced 33 years ago, when only some N-terminal residues were thought to be destabilizing. However, studies over the last three decades have shown that all 20 amino acids of the genetic code can act, in cognate sequence contexts, as destabilizing N-terminal residues. Advantages of the proposed terms include their brevity and semantic uniformity for N-degrons and C-degrons. In addition to being topologically analogous, N-degrons and C-degrons are related functionally. A proteolytic cleavage of a subunit in a multisubunit complex can create, at the same time, an N-degron (in a C-terminal fragment) and a spatially adjacent C-degron (in an N-terminal fragment). Consequently, both fragments of a subunit can be selectively destroyed through attacks by the N-degron and C-degron pathways.

degron | proteolysis | ubiquitin | proteasome | N-end rule

The lifespans of protein molecules in a cell range from less than a minute to many days. Regulated protein degradation protects cells from misfolded, aggregated, or otherwise abnormal proteins, and also controls the levels of proteins that evolved to be short-lived in vivo. Some proteolytic pathways can selectively destroy a specific subunit of a protein complex. Such pathways can act as protein-remodeling devices (1). They can either activate or inactivate a protein machine, change its enzymatic specificity, alter its subunit composition, or repair an oligomeric complex, for example, by destroying fragments of a cleaved subunit that are still embedded in the complex. This would allow a replacement of the cleaved subunit by its intact counterpart. Many biological transitions involve remodeling of protein complexes through subunit-selective degradation, in settings that range from cell-division cycles and circadian circuits to cell differentiation and responses to stresses.

One function of protein degradation is the quality control of nascent and newly formed proteins. Selective proteolysis eliminates those proteins (including mutant ones) that fold too slowly, misfold, or do not satisfy other requirements of quality control. Most proteins function as multisubunit complexes, which often assemble cotranslationally. Quality-control systems destroy subunits that are either overproduced relative to other subunits of a complex or do not become incorporated into a complex rapidly enough. The intracellular protein degradation is mediated largely by the ubiquitin (Ub)-proteasome system (UPS) and by autophagy-lysosome pathways, with molecular chaperones being a part of both systems (1–14).

The UPS comprises a set of pathways that have in common two classes of enzymes: E3-E2 Ub ligases and deubiquitylases (DUBs). A Ub ligase recognizes a substrate protein through its degradation signal (degron) (15) and conjugates Ub, a 9-kDa protein (usually in the form of a poly-Ub chain), to an amino acid residue (usually an internal lysine) of the targeted substrate (*SI Appendix*, Fig. S1*A*). DUBs deubiquitylate Ub-conjugated proteins and

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edit poly-Ub chains. In addition, DUBs produce free Ub through cleavages of Ub precursors encoded by Ub genes (1–9).

Most UPS pathways also involve a multisubunit, ATP-dependent protease called the 26S proteasome (*SI Appendix*, Fig. S1*A*). This protease binds to a ubiquitylated protein substrate through a substrate-linked poly-Ub chain, unfolds the protein using proteasome's ATPases (often with involvement of the Cdc48/p97 unfoldase), and processively destroys the protein to ∼10-residue peptides (16–22).

Some UPS pathways have nonproteolytic functions as well. A mammalian genome encodes more than 800 E3 Ub ligases, which target, in general, different degrons. The multitude and diversity of Ub ligases underlie the immense functional reach of UPS. Its pathways participate in just about every physiological process in all eukaryotes, play major roles in aging, and are involved in causation of many diseases, from impairments of immunity to cancer and neurodegeneration.

Terminology for Proteolytic Pathways That Target N-Termini and C-Termini

Degradation signals are features of proteins that make them short-lived in vivo. Such signals determine, in particular, the specificity of UPS. The problem of degradation signals preceded the onset of Ub studies in the 1980s, and remained a mystery until 1986, when the first degradation signals, later termed degrons (15), were discovered at the N-termini of short-lived proteins, an advance made possible by the invention of the Ub fusion technique (*SI Appendix*, Fig. S1*B*) (23–26). A set of these N-terminal (Nt) signals, later called N-degrons (15), was referred to by the term "N-end rule," which related the in vivo half-life of a protein to the identity of its Nt-residue (23–27). Studies over the next three decades identified proteolytic systems that recognize distinct classes of N-degrons and destroy, often conditionally, specific proteins or their natural fragments that bear N-degrons (Figs. 1 and 2 and *SI Appendix*, Fig. S2*A*) (22–41). Other studies, in the 1990s and afterward, have also identified many internal degrons, defined as degradation signals whose functionally essential elements do not include either Nt-residues or C-terminal (Ct) residues.

The first example of physiologically relevant Ct-degradation signals, called C-degrons below, was identified in 1996. A specific RNA (SsrA) can terminate, *in trans*, a stalled translation of a bacterial protein while tagging the released protein with the Ct-sequence ANDENYALAA. This segment acts as a C-degron, targeting a protein for degradation by the proteasome-like bacterial protease ClpXP (42). In 2018, the laboratories of Elledge and Yen discovered a remarkably large set of diverse natural C-degrons in human proteins (*SI Appendix*, Fig. S3) (43–45). While differing from N-degrons mechanistically and location-wise, C-degrons are topologically analogous to N-degrons. Specific C-degrons and N-degrons can also be associated functionally through their coformation upon a proteolytic cut, as described below.

In 1986, only some Nt-residues were thought to be destabilizing (23). However, later studies by our laboratory showed that every one of the 20 amino acids in the genetic code can act, in cognate sequence contexts, as a destabilizing Nt-residue of an N-degron (Figs. 1 and 2 and *SI Appendix*, Fig. S2*A*) (10, 23–26, 35, 38, 39, 46–48). The term N-end rule and its definition, cited above, are not commensurate with involvement of the entire gamut of Nt-residues in protein degradation. This understanding, as well as benefits of accurate notations, is the reason for renaming N-end rule pathways as "N-degron pathways." Advantages of this terminology include a recall of both the N-terminus ("N") and degradation ("degron"), and the ease of extending this notation from N-degrons to C-degrons.

In sum, proteolytic systems that target N-degrons are proposed to be called the Arg/N-degron pathway, the Pro/N-degron pathway, and the Ac/N-degron pathway in eukaryotes, the fMet/N-degron pathway in eukaryotes and bacteria, and the Leu/N-degron pathway in bacteria (Fig. 1 and *SI Appendix*, Fig. S2*A*). The prefixes Arg, Pro, Ac, fMet, and Leu specify each pathway by highlighting their unique features, for example, the step of Nt-arginylation as a part of the Arg/N-degron pathway (Fig. 1*G* and *SI Appendix*, Fig. S2*A*).

A Ub ligase of an N-degron pathway can contain several degron-recognizing sites. Such a ligase can bind not only to N-degrons but also to internal degradation signals in proteins that lack an N-degron (26). In the proposed terminology, a substrate of, for example, the Arg/N-degron pathway (Fig. 1*G* and *SI Appendix*, Fig. S2*A*) can be called an Arg/N-degron substrate or an Arg/N-d substrate. Another protein, recognized by the same Ub ligase through a protein's internal degron, can be denoted as an Arg/N-id substrate: that is, a substrate bearing an internal degron of the Arg/N-degron pathway.

N-Degron Pathways of Protein Degradation

The N-degron pathways (formerly "N-end rule pathways") comprise a set of proteolytic systems whose unifying feature is their ability to recognize proteins containing N-degrons, thereby causing the degradation of these proteins by the 26S proteasome or autophagy in eukaryotes and by the proteasome-like ClpAP protease in bacteria (Fig. 1 and *SI Appendix*, Figs. S2*A* and S4) (13, 23, 24, 26, 28, 30–38, 40, 46, 49–54). The main determinant of an N-degron is a destabilizing Nt-residue of a protein. In eukaryotes, an N-degron includes an internal lysine (or lysines) of a substrate protein that acts as the site of polyubiquitylation.

Initially, most N-degrons are pro–N-degrons. They are converted to N-degrons either constitutively (e.g., during the emergence of a protein from a ribosome) or conditionally, via regulated steps. Among the routes to N-degrons are cleavages of proteins by proteases that can expose a destabilizing Nt-residue (29, 55–57). An exopeptidase, for example the mammalian Dpp9 aminopeptidase (it removes dipeptides from N-termini), can convert a pro–N-degron at the N-terminus of a specific protein, such as the Syk kinase, to an N-degron (58). The Dpp9 aminopeptidase, Met-aminopeptidases (they remove Nt-Met from some nascent proteins) (*SI Appendix*, Fig. S1*D*), and endoproteases that include caspases, separases, calpains, and cathepsins, have all been shown to generate N-degrons in vivo through their cleavages of intracellular proteins (26, 29, 55–59). Operationally, these proteases are components of N-degron pathways.

A different and mutually nonexclusive route to N-degrons is through enzymatic Nt-modifications of proteins, including Nt-acetylation, Nt-deamidation, Nt-arginylation, Nt-leucylation, and Nt-formylation of the α-amino groups of Nt-residues (Fig. 1 and *SI Appendix*, Fig. S2*A*). Recognition components of N-degron pathways are called N-recognins. They are either specific E3 Ub ligases or other proteins that can target N-degrons (Figs. 1 and 2 and *SI Appendix*, Figs. S2*A* and S4). All 20 amino acids of the genetic code can act, in cognate sequence contexts, as destabilizing Nt-residues (Fig. 1*A*). Consequently, many proteins in a cell are conditionally short-lived N-degron substrates, either as full-length proteins or as protease-generated Ct-fragments (29, 35, 47, 55–57).

Selective degradation of proteins or their natural fragments by N-degron pathways has been shown to regulate a multitude of processes, including: the sensing of oxygen, nitric oxide (NO), heme, and short peptides; the control of subunit stoichiometries in protein complexes; the elimination of misfolded or otherwise abnormal proteins; the degradation of proteins that are retrotranslocated to the cytosol from other compartments, such as mitochondria; the regulation of apoptosis and repression of neurodegeneration; the regulation of DNA repair, transcription, replication, and chromosome cohesion/segregation; the regulation of G proteins, cytoskeletal proteins, autophagy, gluconeogenesis, peptide transport, meiosis, immunity, circadian rhythms, fat metabolism, cell migration, cardiovascular development, spermatogenesis, and neurogenesis; and the regulation of leaf and shoot development, oxygen/NO sensing, and many other

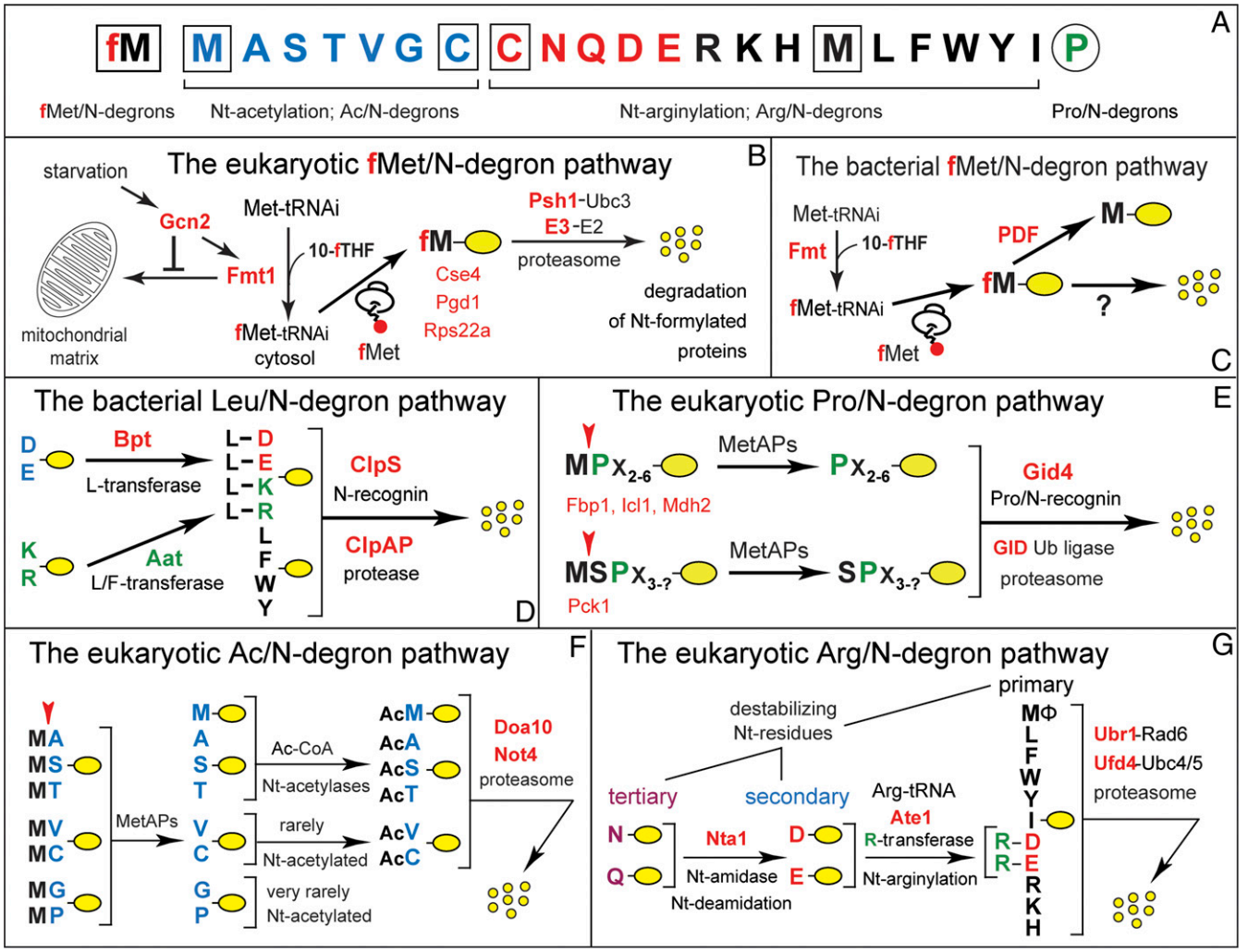

Fig. 1. N-degron pathways. Nt-residues are indicated by single-letter abbreviations. A yellow oval denotes the rest of a protein substrate. (*A*) Twenty amino acids of the genetic code are arranged to delineate specific N-degrons. Nt-Met is cited three times because it can be recognized by the Ac/N-degron pathway (as Nt-acetylated Ac-Met), by the Arg/N-degron pathway (as unacetylated Nt-Met), and by the fMet/N-degron pathway (as Nt-formylated fMet). Nt-Cys is cited twice, because it can be recognized by the Ac/N-degron pathway (as Nt-acetylated Cys) and by the Arg/N-degron pathway (as an oxidized, arginylatable Nt-Cys sulfinate or sulfonate, formed in multicellular eukaryotes but apparently not in unstressed *S. cerevisiae*). (*B*) The eukaryotic (*S. cerevisiae*) fMet/N-degron pathway (39); 10-fTHF, 10-formyltetrahydrofolate. (*C*) The bacterial (*E. coli*) fMet/N-degron pathway (38). (*D*) The bacterial (*V. vulnificus*) Leu/N-end rule pathway (51). (*E*) The eukaryotic (*S. cerevisiae*) Pro/N-degron pathway (35–37). (*F*) The eukaryotic (*S. cerevisiae*) Ac/N-degron pathway (10, 46–48). (*G*) The eukaryotic (*S. cerevisiae*) Arg/N-degron pathway (26, 31). Modified with permission from ref. 38.

processes in plants (see refs. 26, 30–35, 48, 56, 57, and 59 and references therein).

The field of N-degrons and C-degrons is too large for a comprehensive review in a Perspective-size article. Instead of describing all pathways equally briefly, the Arg/N-degron pathway is discussed below in relative detail, followed by much shorter accounts of other pathways.

The Arg/N-Degron Pathway

This eukaryotic pathway targets unacetylated Nt-residues (Figs. 1*G* and 2*B* and *SI Appendix*, Figs. S2*A* and S4) (23, 55, 59). Nt-Arg, -Lys, -His, -Leu, -Phe, -Tyr, -Trp, -Ile, and -Met (if Nt-Met is followed by a bulky hydrophobic residue) are directly recognized by Arg/N-recognins. Examples of Arg/N-recognins include: the *Saccharomyces cerevisiae* Ubr1 E3; the mammalian Ubr1, Ubr2, Ubr4, and Ubr5 E3s; the Prt1 and Prt6 E3s of plants; and the mammalian non-E3 autophagy regulator p62/Sqstm1 (Fig. 1*G* and *SI Appendix*, Figs. S2*A* and S3) (13, 26, 32, 33, 47, 49, 50). The Nt-Asn, -Gln, -Glu, and -Asp residues (as well as Nt-Cys, under some conditions) are destabilizing because of enzymatic deamidation of Nt-Asn and -Gln, and Nt-arginylation of Nt-Asp, -Glu, and (oxidized) -Cys (Fig. 1*G* and *SI Appendix*, Fig. S2*A*) (40, 60–62).

Double-E3 Design of the Arg/N-Degron Pathway. Ubr1 is the sole Arg/N-recognin in *S. cerevisiae*, but the pathway's targeting complex contains two E3s: the 225-kDa RING-type Ubr1 and the 168-kDa HECT-type Ufd4, in association with their respective E2 enzymes Rad6 and Ubc4/Ubc5 (63) (Fig. 1*G*). The Ubr1-bound Ufd4 increases the processivity of polyubiquitylation (63). In contrast to Ubr1, Ufd4 is not an Arg/N-recognin. Specifically, Ufd4 does not, by itself, recognize Arg/N-degrons. However, Ufd4 can bind to substrate proteins such as Mgt1, Cup9, and Chk1, through their internal degrons that are also recognized by Ubr1 (63, 64). Exactly how the recognition of an internal degron by both Ubr1 and Ufd4 is achieved within the Ubr1–Ufd4 complex (do Ubr1 and Ufd4 compete for the same elements of a degron, or did

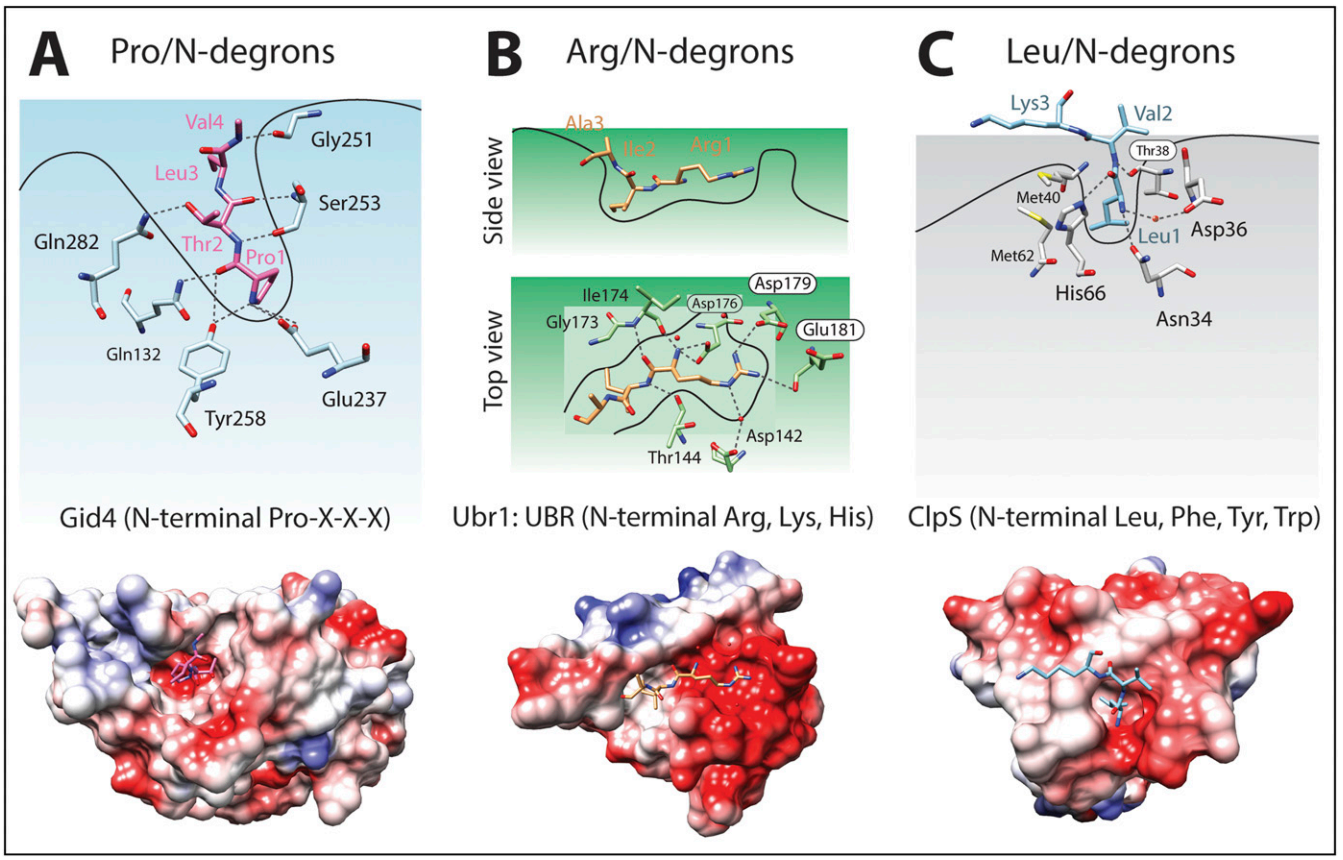

Fig. 2. Structural basis of N-degron recognition. The upper diagrams schematically depict the substrate-binding sites of different N-recognins, with corresponding space-filling images indicating electrostatic potential (red, negative; blue, positive) below the diagrams. (*A*) The substrate-binding site of the human Gid4 Pro/N-recognin (35–37). (*B*) One of substrate-binding sites (the UBR box, which recognizes basic Nt-residues) of the *S. cerevisiae* Ubr1 Arg/N-recognin. (*C*) The substrate-binding site of the *E. coli* ClpS Leu/N-recognin, which recognizes bulky hydrophobic Nt-residues (30, 52, 53). Modified with permission from ref. 35.

these E3s evolve to recognize a cognate degron simultaneously?) remains to be understood.

Substrates of the Arg/N-Degron Pathway and Their Protection by Chaperones. A molecule of a protein, including a newly formed protein, would be longer-lived if that molecule succeeds, rapidly enough, to become a subunit of a "protective" complex, often a cognate complex in which that subunit normally functions. Stabilization of the subunit would be caused by steric shielding of its degrons within the complex (10). A protection can also be attained through the binding of a vulnerable protein to a molecular chaperone, particularly the Hsp90 system, which comprises Hsp90 and more than 10 of its cochaperones. Hsp90 reversibly binds to at least 20% of cellular proteins, called Hsp90 clients, including most kinases and transcriptional regulators. Hsp90 assists its clients, often repeatedly, in maintaining their active conformations (14).

Oh et al. (64) showed that a weakening of the *S. cerevisiae* Hsp90 system (i.e., an increase in the fraction of Hsp90 clients that are not bound to Hsp90), causes many otherwise long-lived proteins to become short-lived, because of their rapid degradation by the Arg/N-degron pathway. Diverse Hsp90 clients, including Chk1, Kar4, Tup1, Gpd1, Ste11, and also, remarkably, Hsp82 [i.e., the Hsp90 chaperone itself (suggesting that Hsp90 is its own client)], become short-lived substrates of the Arg/N-degron pathway under conditions of hypoactive Hsp90 (64). The cited proteins are targeted by Ubr1/Ufd4 through their internal degrons (64). The Arg/N-degron pathway has also been shown to destroy a variety of misfolded proteins (reviewed in refs. 26, 31, and 34).

Mammalian Ubr1, Ubr2, Ubr4, and Ubr5. In contrast to *S. cerevisiae*, in which the Ubr1 E3 is the sole Arg/N-recognin (Figs. 1*G* and 2*B*), a mammalian genome encodes at least four E3s that can recognize Arg/N-degrons: the 200-kDa Ubr1 and Ubr2, the 570-kDa Ubr4 (p600; Big), and the 300-kDa Ubr5 (Edd1; Hyd) (31) (*SI Appendix*, Fig. S2*A*). Ubr1 and Ubr2 are highly sequelogous (similar in sequence) (65) to each other and to *S. cerevisiae* Ubr1 (26, 31). In contrast, the sequelogy (sequence similarity) (65) between, for example, Ubr1 and either Ubr4 or Ubr5, is largely confined to their ~80-residue UBR domains. ["Sequelog" denotes a sequence that is similar, to a specified extent, to another sequence (65). Derivatives of sequelog include "sequelogous" (similar in sequence) and "sequelogy" (sequence similarity). The usefulness and appeal of sequelog and derivative terms stem from the rigor of their evolutionary neutrality. In contrast, the terms "homolog," "ortholog," and "paralog," which invoke, respectively, common descent and functional similarity or dissimilarity, are interpretation-laden and often less than precise notations. Homolog, ortholog, and paralog are compatible with the sequelog terminology, and can be used to convey understanding about common descent and biological functions, if this additional information (it is distinct from sequence similarities per se) is actually present (65).]

Ubr4, a huge (570 kDa) Arg/N-recognin, functions, in particular: in neurogenesis; in cell migration; in the biogenesis of endosomes; in cardiovascular development and autophagy; in the degradation of

podocin, a protein that maintains the renal filtration barrier; and in auxin transport in plants (ref. 66 and references therein). The functions of the Ubr5 Arg/N-recognin include: regulation of Wnt/β-catenin; the degradation of huntingtin, hPXR, Gkn1, and many other proteins; and also specific roles as either an oncoprotein or a tumor suppressor (ref. 67 and references therein). Connections between the functions of Ubr4/Ubr5 and their ability to recognize Arg/N-degrons remain to be understood.

Johanson-Blizzard Syndrome. Johanson-Blizzard Syndrome (JBS) patients lack Ubr1 but retain other Arg/N-recognins, including Ubr2, a sequelog (65), and functional analog of Ubr1 (*SI Appendix*, Fig. S2*A*). Symptoms of JBS include an exocrine pancreatic insufficiency and inflammation, multiple malformations (e.g., a near-absence of nasal wings), as well as mental retardation and deafness (refs. 26 and 68 and references therein). *Ubr1*$^{-/-}$ mice exhibit JBS symptoms in a milder form. Mice lacking Ubr2 have other defects, including infertility in males (because of apoptosis of spermatocytes) and genomic instability (31). In contrast to viability of *Ubr1*$^{-/-}$ and *Ubr2*$^{-/-}$ mouse strains, double-mutant mice, lacking both Ubr1 and Ubr2, die as midgestation embryos, with defects in neurogenesis and cardiovascular development (refs. 26 and 31 and references therein).

Structure and Targeting of Arg/N-Degrons. The main determinant of an Arg/N-degron is a substrate's specific Nt-residue (Fig. 1*G* and *SI Appendix*, Figs. S2*A* and S5). Once an Arg/N-recognin (as a part of a targeting complex) binds to a destabilizing Nt-residue of a substrate, a race against time begins, given the transience of the bound state and the necessity, for a successful targeting, to produce a substrate-linked poly-Ub chain. The synthesis of a proteasome-binding poly-Ub chain is initiated at an internal lysine of a substrate. This lysines is the second determinant of an Arg/N-degron (*SI Appendix*, Fig. S5) (1, 22, 24, 26, 31). The third determinant of an Arg/N-degron is an unstructured segment that the substrate-bound proteasome uses to initiate proteolysis (21, 22, 24, 26).

Subunit Selectivity of Protein Degradation. The Arg/N-degron pathway can destroy a subunit of a complex while sparing the rest of the complex (1, 26). Subunit selectivity, discovered in 1990, involved in addition the discovery of *trans*-targeting (*SI Appendix*, Fig. S5) (1). In this process, an Arg/N-recognin binds to a destabilizing Nt-residue of a subunit that lacks an efficacious second-determinant lysine. It was found that the subunit-bound Arg/N-recognin could polyubiquitylate *in trans* another subunit of the same complex (if it contained a "suitable" second-determinant lysine), and thereby would target for degradation specifically that subunit rather than the initially bound one. In sum, the multideterminant organization of an Arg/N-degron allows it to be "split" between subunits of a complex, leading to a targeting *in trans* (*SI Appendix*, Fig. S5) (1, 26).

Physiological Arg/N-Degron Substrates. The list of physiological Arg/N-degron substrates is already large and continues to grow (*SI Appendix*, Figs. S6–S8). An example of Arg/N-degron substrates that are not cited in *SI Appendix*, Figs. S6–S8 is Phe-Pink1, a Ct-fragment of the Pink1 kinase. Pink1 is imported into mitochondria and is conditionally cleaved there. The Youle laboratory (see ref. 69 for review) showed that the Phe-Pink1 Ct-fragment is retrotranslocated to the cytosol and is destroyed by the Arg/N-degron pathway. Pink1 phosphorylates, in particular, the E3 Ub ligase parkin and Ub itself. Null mutations in both copies of human *PINK1* result in early-onset Parkinson disease, a neurodegeneration syndrome. The uncleaved Pink1 accumulates in the outer mitochondrial membrane (OMM) and recruits parkin to OMM, a step that can lead to an autophagosome-mediated engulfment of damaged mitochondria and their destruction in lysosomes. Generation and degradation of the Phe-Pink1 Ct-fragment are a part of circuits that regulate the levels of OMM-bound uncleaved Pink1 and mitochondrial quality control (ref. 69 and references therein).

Roq1 as a Substrate and Regulator of the Arg/N-Degron Pathway. A natural Ct-fragment of *S. cerevisiae* Roq1 acts as both a substrate and regulator of the Arg/N-degron pathway (41). Tunicamycin, a drug that causes protein misfolding in the endoplasmic reticulum (ER), increases the level of *ROQ1* mRNA. An artificial increase of *ROQ1* mRNA can accelerate the degradation, by the Arg/N-degron pathway, of an ER membrane-embedded reporter protein. The Ynm3 endoprotease can cleave Roq1, generating the Arg-Roq1 Ct-fragment. This cleavage of Roq1 is required for the accelerated degradation of the above reporter. Arg-Roq1 is destroyed, in part, by the Arg/N-degron pathway (41). Remarkably, interactions between Arg-Roq1 and Ubr1 can alter the targeting efficacy of Ubr1 toward its other substrates, such as, for example, Cup9, which bears an internal degron. One possibility is that Arg-Roq1 modulates the specificity and efficacy of Ubr1 under conditions of stress (41).

Accelerators of Apoptosis as Arg/N-Degron Substrates. During apoptosis, caspases cleave more than 1,000 different proteins in a mammalian cell. Caspase-mediated cleavages of cellular proteins can generate proapoptotic Ct-fragments, defined as those that increase the probability of apoptosis. Such Ct-fragments often bear destabilizing Nt-residues (*SI Appendix*, Fig. S6). It was found that the natural proapoptotic Ct-fragments Cys-Ripk1, Cys-Traf1, Asp-Brca1, Leu-Likk1, Tyr-Nedd9, Arg-Bid, Asp-Bcl$_{XL}$, Arg-Bim$_{EL}$, Asp-Epha4, and Tyr-Met were short-lived substrates of the Arg/N-degron pathway (*SI Appendix*, Fig. S6) (refs. 34 and 55 and references therein). In agreement with these results, even a partial ablation of the Arg/N-degron pathway sensitizes cells to apoptosis (55). In sum, the Arg/N-degron pathway is a regulator of apoptosis, acting largely (but not necessarily exclusively) (34) as an antiapoptotic circuit (55). By destroying proapoptotic Ct-fragments, the Arg/N-degron pathway contributes to thresholds that prevent a transient or otherwise weak proapoptotic signal from reaching the point of commitment to apoptosis.

Ubr1 Binds to Caspases. Weaver et al. (70) showed that the Ubr1 Arg/N-recognin of the nematode *Caenorhabditis elegans* binds to both the procaspase Ced3 and its proteolytically activated form. One substrate of Ced3 is Lin28, a regulator of cell differentiation. Activated Ced3 cleaves Lin28, generating its Nt-Asn–bearing Ct-fragment that is rapidly destroyed by the Arg/N-degron pathway. In *ubr1Δ* worms the level of Lin28 was increased (as would be expected), but Lin28 was also at most weakly cleaved by Ced3 (70). The latter finding suggested that Ubr1 not only mediates the degradation of the caspase-generated Asn^{31}-Lin28, but may also activate the Ced3 procaspase. If so, the Arg/N-degron pathway might be a previously unknown route for activation of caspases, a most interesting possibility.

Regulation of Peptide Transport by the Arg/N-Degron Pathway. In the absence of extracellular di/tripeptides, the *S. cerevisiae* transcriptional repressor Cup9 shuts off (nearly but not entirely) the *PTR2* gene, which encodes the transmembrane peptide importer. This makes cells nearly (but not entirely) incapable of importing di/tripeptides (*SI Appendix*, Fig. S9). The type 1 and type 2 binding sites of Ubr1 recognize Arg/N-degrons through their binding, respectively, to basic and bulky hydrophobic Nt-residues in either proteins or short peptides (26, 63, 71).

If a cell finds itself in the presence of extracellular di/tripeptides, they are imported inefficiently at first, because of low initial levels of the Ptr2 transporter. However, imported di/tripeptides

that bear destabilizing Nt-residues can bind to the type 1/2 sites of Ubr1. These interactions activate, allosterically, a separate (third) binding site of Ubr1, the one that recognizes an internal degron of the Cup9 repressor (*SI Appendix*, Fig. S9) (26, 71). The resulting "activated" form of Ubr1 targets Cup9 for degradation, reducing its half-life to ∼1 minute and its levels to negligible. As a result, *PTR2* is derepressed and the Prt2 transporter is overproduced, greatly increasing the capacity of cells to import di/tripeptides (*SI Appendix*, Fig. S9) (26, 71). This positive-feedback circuit enables both the budding yeast *S. cerevisiae* and the fission yeast *Schizosaccharomyces pombe* to detect the presence of extracellular di/tripeptides and to react by accelerating their uptake (71, 72).

Deamidation of Nt-Asn and -Gln. In *S. cerevisiae*, Nt-deamidation is mediated by the 52-kDa Nta1 Nt-amidase (Fig. 1*G*) (40, 73). Remarkably, the bulk of Nta1 is located in the inner mitochondrial matrix (https://yeastgfp.yeastgenome.org/). Nevertheless, a low cytosolic (and presumably nuclear) level of Nta1 suffices to mediate the Arg/N-degron pathway (40, 73). Physiological substrates of yeast Nta1 remain to be discovered. Mitochondrial Nta1 might be a component of a distinct (still to be identified) N-degron pathway in the mitochondrial matrix (26).

In animals and plants, Nt-deamidation is mediated by the *Ntan1*-encoded, Nt-Asn–specific Nt^N-amidase and the *Ntaq1*-encoded, Nt-Gln–specific Nt^Q-amidase (*SI Appendix*, Fig. S2*A*) (74). Ntan1 and Ntaq1 are present in the cytosol/nucleus, in contrast to the largely mitochondrial *S. cerevisiae* Nta1 (ref. 74 and references therein). In the fly *Drosophila melanogaster*, the cleavage, by a caspase, of the antiapoptotic Ub ligase Diap1 generates the short-lived Asn^{21}-Diap1 Ct-fragment that is much less efficacious than full-length Diap1 in repressing apoptosis. Degradation of Asn^{21}-Diap1 requires Ntan1 (*SI Appendix*, Fig. S2*A*) (75). A virus would benefit from a delay of apoptosis, as this would facilitate the completion of viral replication in an infected cell. Remarkably, a picorno-like RNA virus induces, through an unknown mechanism, the proteasome-dependent degradation of the Ntan1 Nt^N-amidase in infected insect cells, resulting in a partial stabilization of Asn^{21}-Diap1 (76). This way, a viral infection can down-regulate apoptosis, benefiting the virus (76).

Nt-Arginylation. The 60-kDa Ate1 R-transferase catalyzes the conjugation of Arg (provided by Arg-tRNA) to the α-amino group of a specific Nt-residue of a protein. The resulting Nt-Arg can be bound by Arg/N-recognins (Fig. 1*G* and *SI Appendix*, Figs. S2 and S4). In mammals, there are six isoforms of R-transferase, produced through alternative splicing of the *Ate1* pre-mRNA (*SI Appendix*, Fig. S2 *B* and *C*) (ref. 26 and references therein). A number of natural Ct-fragments, including nearly full-length proteins, are either confirmed or putative substrates of the Ate1 R-transferase and the rest of the Arg/N-degron pathway (*SI Appendix*, Figs. S6–S8).

Arginylation and the Sensing of Oxygen and NO. In 2005, it was discovered that the mammalian Arg/N-degron pathway is a new kind of oxygen (O_2) and NO sensor. The NO/O_2-dependent oxidation of Nt-Cys converts it to Nt-Cys-sulfinate or Nt-Cys-sulfonate, which can be Nt-arginylated, in contrast to unmodified Nt-Cys (*SI Appendix*, Fig. S2*A*) (60, 61). The NO/O_2-dependent proteolysis by the Arg/N-degron pathway controls the levels of a subset of proteins that bear Nt-Cys, including Rgs4, Rgs5, and Rgs16 (60, 61). These conditionally short-lived proteins are regulators of specific G proteins.

The Arg/N-degron pathway is also the main sensor of NO/O_2 in plants, through the NO/O_2-dependent oxidation of Nt-Cys in conditionally short-lived transcription factors that include Rap2.12, Rap2.2, Rap2.3, Hre1, and Hre2 (33, 77, 78). In plants, and possibly in other multicellular eukaryotes as well, the NO/O_2-dependent oxidation of Nt-Cys is catalyzed by Cys-oxidases, in addition to a nonenzymatic oxidation of Nt-Cys. In vivo levels of the above transcription factors and the expression of regulons controlled by them underlie adaptations to a broad range of stresses experienced by plants (refs. 32, 33, 77, and 78 and references therein).

The Arg/N-Degron Pathway as a Sensor of Heme. Both mammalian and yeast Ate1 R-transferases are inhibited by low micromolar levels of hemin (Fe^{3+}-heme) (79). Hemin also accelerates, in vivo, the degradation of mouse Ate1, thereby acting as both a "stoichiometric" and "catalytic" down-regulator of Nt-arginylation. Thus, in addition to being a sensor of NO, O_2, and short peptides, the Arg/N-degron pathway is also a sensor of heme (*SI Appendix*, Fig. S2*A*) (79).

Arginylation, Autophagy, and the Arg/N-Degron Pathway. Kwon and colleagues (13, 49, 50) discovered that p62/Sqstm1 (called p62 below), a component of the autophagy-lysosome system, is also a non-E3 Arg/N-recognin that binds to cytosolic proteins that bear either Nt-Arg or specific hydrophobic Nt-residues. p62 mediates the capture of these proteins by autophagy and their subsequent destruction in lysosomes (refs. 12, 13, 49, and 50 and references therein). Either a proteasome inhibitor or natural stresses can up-regulate the p62/autophagy branch of the Arg/N-degron pathway, termed the Arg/N-$degron^{p62}$ pathway (*SI Appendix*, Fig. S4) (12, 13, 49, 50).

BiP (one of Hsp70 chaperones), calreticulin (another ER chaperone), and protein disulfide isomerase are among ER-resident proteins that bear Nt-arginylatable Nt-residues, such as Nt-Asp or Nt-Glu. Upon stresses, including heat shock and unfolded protein response, a fraction of these ER proteins is transferred to the cytosol, followed by their Nt-arginylation. The resulting Nt-Arg–bearing proteins are captured either by the p62 Arg/N-recognin or by E3 Arg/N-recognins, and are destroyed by the autophagy-lysosome system (the Arg/N-$degron^{p62}$ pathway) or by the 26S proteasome (*SI Appendix*, Fig. S4) (49, 50). In sum, the Arg/N-degron pathway is a major functional link between UPS and autophagy (refs. 12, 13, 49, and 50 and references therein).

The Ac/N-Degron Pathway

About 60% and more than 80% of, respectively, *S. cerevisiae* and human proteins are irreversibly N^{α}-terminally acetylated (Nt-acetylated) by Nt-acetylases (80). The 2010 discovery of Ac/N-degrons (46) identified a major function of Nt-acetylation, a universally present modification whose significance was, until then, largely obscure. The Ac/N-degron pathway targets proteins for degradation by recognizing their Nt-acetylated Nt-residues (Fig. 1*F*) (10, 46–48). The E3 Ub ligases (Ac/N-recognins) of this pathway are the ER membrane-embedded yeast Doa10 and its mammalian counterpart Teb4, and also Not4, the E3 subunit of Ccr4-Not, a multifunctional cytosolic/nuclear complex (10, 46, 48).

Schulman and coworkers (81) showed that the Nt-Ac group of a subunit in a protein complex usually increases thermodynamic stability of the complex. The affinity-enhancing effect of Nt-acetylation provides an explanation for at least intermittently long half-lives of many Nt-acetylated proteins. Specifically, natural Ac/N-degrons tend to be conditional, because of their rapid sequestration within cognate protein complexes (10). The functions of the Ac/N-degron pathway (Fig. 1*F*) include quality control and the regulation of input protein stoichiometries in vivo. For example, *S. cerevisiae* Nt-Ac-Cog1, a short-lived Ac/N-degron substrate, can be made long-lived by coexpressing Cog2 or Cog3, the Cog1-binding subunits of the Golgi-associated COG complex (10). Analogously, *S. pombe* Nt-Ac-Hcn1, a short-lived Ac/N-degron substrate, can be stabilized by coexpressing Cut9, a cognate ligand of Hcn1 in the APC/C Ub ligase (10).

The Pro/N-Degron Pathway

When glucose is low or absent, cells synthesize it through gluconeogenesis. In yeast, the main gluconeogenesis-specific cytosolic enzymes are the Fbp1 fructose-1,6-bisphosphatase, the Icl1 isocitrate lyase, the Mdh2 malate dehydrogenase, and the Pck1 phosphoenolpyruvate carboxykinase. When *S. cerevisiae* grows on a nonfermentable carbon source such as, for example, ethanol, the gluconeogenic enzymes are expressed and long-lived. Transition to a medium containing glucose inhibits the synthesis of these enzymes and induces their degradation, mediated by the multisubunit GID Ub ligase and the proteasome (ref. 35 and references therein).

We discovered that Gid4, a subunit of GID, is the N-recognin of a proteolytic system termed the Pro/N-degron pathway (Figs. 1*E* and 2*A*) (35). Gid4 recognizes a substrate through its Nt-Pro residue or a Pro at position 2, in the presence of distinct (but nonunique) adjoining sequence motifs. The gluconeogenic enzymes Fbp1, Icl1, Mdh2, and Pck1 bear either Nt-Pro or a Pro at position 2, and are conditionally short-lived substrates of the Gid4-dependent Pro/N-degron pathway (Fig. 1*E*) (35–37). The structure of Gid4 comprises an antiparallel β-barrel that contains a deep and narrow substrate-binding cleft (Fig. 2*A*) (36, 37).

The Eukaryotic fMet/N-Degron Pathway

Nascent proteins bear Nt-Met, encoded by the AUG initiation codon. In bacteria and in eukaryotic organelles, mitochondria, and chloroplasts, formyltransferases Nt-formylate the Met moiety of initiator Met-tRNAs. Consequently, nascent bacterial proteins start with Nt-fMet. In contrast, proteins synthesized by the cytosolic ribosomes of eukaryotes bear unformylated Nt-Met, which is often cotranslationally Nt-acetylated, resulting in Ac/N-degrons (Fig. 1*F*) (10, 46–48).

In 2015, it was found that Nt-fMet residues of nascent bacterial proteins can act as bacterial N-degrons, termed fMet/N-degrons (Fig. 1*C*) (38). Remarkably, it was recently discovered that Nt-formylation of proteins, previously thought to be confined to bacteria and bacteria-derived eukaryotic organelles, can also occur at the start of translation by the cytosolic ribosomes of a eukaryote, such as *S. cerevisiae* (Fig. 1*B*) (39). Nt-formylation of yeast cytosolic proteins is mediated by the nuclear DNA-encoded Fmt1 formyltransferase, whose translocation from the cytosol to the inner matrix of mitochondria was found to be not as efficacious, even under normal conditions, as had previously been assumed, and is strongly impaired under conditions of stationary phase and other stresses (39). The cytosolic retention of Fmt1, and the resulting upsurge in the levels of Nt-formylated cytosolic proteins in nutritionally stressed cells, require Gcn2, a protein kinase (39). It was also discovered that Nt-formylated cytosolic proteins are targeted for selective degradation by the Psh1 E3 Ub ligase, which acts as the fMet/N-recognin of the previously unknown eukaryotic fMet/N-degron pathway (Fig. 1*B*) (39).

The Bacterial Leu/N-Degron Pathway

The bacterial Leu/N-degron pathway, which does not involve ubiquitylation, was discovered in 1991 (28) and characterized in Gram-negative bacteria (Fig. 1*D*) (refs. 26, 30, and 51–54 and references therein). This pathway comprises the following components: (*i*) ClpAP, a proteasome-like, ATP-dependent protease; (*ii*) ClpS, the 12-kDa Leu/N-recognin that binds to Nt-Leu, -Phe, -Trp, or -Tyr and delivers bound substrates to the ClpAP protease; (*iii*) Aat, an L/F-transferase that employs Leu-tRNA or Phe-tRNA as a cosubstrate to conjugate largely Leu (and occasionally Phe) to the N-termini of proteins bearing Nt-Lys or Nt-Arg (Fig. 1*D*); and (*iv*) Bpt, an L-transferase that employs Leu-tRNA to conjugate Leu to Nt-Asp, -Glu, and (possibly) oxidized -Cys (Fig. 1*D*). *Vibrio vulnificus*, a human pathogen, contains both Aat and Bpt, while *Escherichia coli* contains only Aat (51). Physiological substrates of the *E. coli* Leu/N-degron pathway include Dps, an 18-kDa DNA-binding protein that compacts the *E. coli* nucleoid in starving cells, and the YgjG putrescine-aminotransferase (PATase) (refs. 30 and 52 and references therein). Although *E. coli* ClpS is nearly 20-fold smaller than yeast or human Ubr1, there is a significant sequelogy between the substrate-binding region of ClpS and a functionally analogous region of Ubr1, suggesting a common descent of bacterial and eukaryotic N-recognins (26, 30).

Studies by Groisman and coworkers indicated that ClpS can target not only N-degrons (Fig. 1*D*), but also N-terminus–proximal internal degrons in bacterial proteins, such as PhoP (ref. 54 and references therein). In a pathway that regulates PhoP, the MgtC protein competes with ClpS for the binding to PhoP, and thereby protects PhoP from degradation. In addition, PhoP, a conditionally short-lived substrate of ClpS, is a transcriptional repressor of ClpS expression. The resulting circuits differentially regulate the rates of degradation of specific ClpS substrates under conditions of low intracellular Mg^{2+} (54). Because bacterial ClpS is a sequelog (65) of much bigger eukaryotic Arg/N-recognins E3s, such as Ubr1 (26, 31), the largely unexplored regulation of the Arg/N-degron pathway in yeast and multicellular eukaryotes may prove to be at least as functionally rich as the already revealed regulation of ClpS and the bacterial Leu/N-degron pathway (ref. 54 and references therein).

Eukaryotic C-Degron Pathways

Because of its free carboxyl group, the Ct-residue of a polypeptide is stereochemically unique, analogously to the Nt-residue and its α-amino group. In 2018, the laboratories of Elledge and Yen discovered a remarkably large set of Ct-degradation signals in human proteins (*SI Appendix*, Fig. S3) (43–45). They also showed that specific E3 Ub ligases of the cullin-RING (CRL) family, and other E3s as well, can recognize these degrons (43–45).

The authors' terms for Ct-degradation signals and pathways that recognize them were, respectively, "C-end degrons" and "DesCEND" (destruction via C-end degron) (43, 44). For reasons discussed at the beginning of this paper, we propose to denote "C-end degrons" as "C-degrons," and "DesCEND pathways" as "C-degron pathways" (*SI Appendix*, Fig. S3). In addition to their succinctness as well as semantic uniformity vis-à-vis N-degrons, it is easy to adapt these terms to specific settings. For example, a pathway mediated by the C-degron–recognizing Kldhc3 subunit of the Crl2 Ub ligase (43, 44) can be called the C-degron$^{Kldhc3}_{Crl2}$ pathway.

Functional Aspects of C-degrons. C-degrons can be present in full-length proteins, in truncated proteins that result from premature termination of translation, and in protein fragments that form upon proteolytic cuts (*SI Appendix*, Fig. S3) (43, 44). All such proteins would be afforded, in vivo, a transient stochastic opportunity to fold or associate in ways that would shield their C-degrons. A C-degron–containing polypeptide that fails to shield its C-degron rapidly enough would face the rising probability of destruction by a cognate C-degron pathway. This temporal pattern is universal among C-degrons and other degradation signals, in that it is relevant to any protein whose degron-based susceptibility to a proteolytic attack changes as a function of time, with the clock beginning to tick at the time of protein's emergence from the ribosomal tunnel.

Cocreation of C-Degrons and N-Degrons upon a Proteolytic Cut. Usp1 is a mammalian DUB (82). Usp1 forms a heterodimer with Uaf1, a non-DUB protein (*SI Appendix*, Fig. S10). Usp1 can autocleave immediately after its internal Gly-Gly sequence (82). The resulting Ct-fragment, Gln-Usp1^{Ct}, bears a deamidation/arginylation-dependent Arg/N-degron (*SI Appendix*, Fig. S10) (56). Nevertheless, the DUB activity of autocleaved Usp1 can be transiently maintained, inasmuch as Usp1^{Nt}, the Nt-fragment of autocleaved Usp1, can remain bound

to the Gln-Usp1^{Ct} Ct-fragment within the cleaved Usp1-Uaf1 heterodimer. The Ct-sequence Gly-Gly of the Usp1^{Nt} Nt-fragment can act as a C-degron, which is recognized by the Kldch2 adaptor subunit of the Clr2 Ub ligase (44). In the resulting mechanism (not yet analyzed in detail), Uaf1 would hold together two Usp1 fragments, allowing them to function, temporarily, as a DUB enzyme, until successful attacks on both fragments by the N-degron and C-degron pathways (refs. 56 and 82 and references therein). Usp1 is the first experimentally addressed setting in which an N-degron and a C-degron can be cocreated upon a cleavage (self-cleavage, in this case) of a full-length protein.

Concluding Remarks

In 1984–1990, studies by our laboratory described the discovery of the first degradation signals (N-degrons) in short-lived proteins; the singular biological significance of UPS; the first physiological functions of ubiquitylation, in the cell cycle, DNA repair, protein synthesis, transcriptional regulation, and stress responses; the Arg/N-degron pathway as the first specific UPS pathway; the subunit selectivity of Ub-dependent proteolysis; the first specific poly-Ub chains and their necessity for protein degradation; the Matα2 repressor as the first physiological substrate of UPS; the first nonproteolytic function of Ub (its role as a cotranslational chaperone); and initiated the molecular genetic understanding of UPS, including the cloning of the first E3 Ub ligase (Ubr1), the first DUBs (Ubp1–Ubp3), and the first precursors of free Ub (Ubi1-Ubi4) (refs. 3 and 4 and references therein). Just how broad and elaborate Ub functions are was understood systematically over the next three decades through studies by many laboratories that entered the field in the 1990s and afterward, an expansion that continues to this day.

Studies of N-degron pathways remained a fount of new genetic and biochemical methods for more than three decades, giving rise to the Ub fusion technique, the Ub reference technique, the Ub translocation technique, the split-Ub technique, the Ub sandwich technique, the heat-inducible N-degron (refs. 3, 4, and 25 and references therein), and other methods by other laboratories.

UPS is of major relevance to medicine. Pharmaceutical companies and academic laboratories are developing compounds that target specific UPS components. The fruits of their labors have already become—or will soon become—clinically useful drugs. Work in this arena is producing not only "conventional" inhibitors or activators of specific enzymes, but also drugs that can direct a Ub ligase to target, destroy, and thereby down-regulate any specific protein. Given the broad functional range of N-degron and C-degron pathways, they will be a part of these advances.

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