Targeting Proteins for Destruction by the Ubiquitin System: Implications for Human Pathobiology

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proteasome, proteolysis, degradation, disease

Abstract

Cellular proteins are in a dynamic state maintained by synthesis and degradation. The ubiquitin proteolytic pathway is responsible for the degradation of the bulk of cellular proteins including short-lived, regulatory, and misfolded/denatured proteins. Ubiquitin-mediated proteolysis involves covalent attachment of multiple ubiquitin molecules to the protein substrate and degradation of the targeted protein by the 26S proteasome. Recent understanding of the molecular mechanisms involved provides a framework to understand a wide variety of human pathophysiological states as well as therapeutic interventions. This review focuses on the response to hypoxia, inflammatory diseases, neurodegenerative diseases, and muscle-wasting disorders, as well as human papillomaviruses, cervical cancer and other malignancies.

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INTRODUCTION

This review focuses on the targeting and degradation of intracellular protein substrates via the ubiquitin-proteasome system and its association with human diseases. The recent rapid expansion in our knowledge of the molecular components and mechanisms of action of the ubiquitin-proteasome system provides both a framework for understanding key regulatory steps in a wide variety of pathophysiological states, as well as strategies for therapeutic intervention. As several recent reviews have focused on the molecular components of the ubiquitin-proteasome system (1–8), herein we primarily address issues related to pathogenesis and potential therapeutics. We include discussions related to the response to hypoxia, inflammatory diseases, neurodegenerative diseases, and muscle-wasting disorders, as well as human papillomaviruses, cervical cancer, and other malignancies.

The body's proteins are in a dynamic state (9). Under physiological conditions in the human adult, whole body protein degradation is approximately equal to whole body protein synthesis. Using [^{15}N]glycine, estimates of protein turnover yield ~4 g/kg body weight per day or ~2% per day (10). Similar results were derived from estimates of leucine production. Protein turnover (per kg body weight) is more rapid in infants and children and is almost four times faster in premature infants (11), whereas older adults have slower protein turnover than young adults (12).

Rates of protein degradation vary also among organs (e.g., kidney proteins turn over >10–20 times faster than skeletal muscle proteins) (13). Although muscle proteins constitute >50% of body protein mass, protein turnover accounts for only ~30% of whole body turnover (14). Myofibrillar proteins represent ~66% of muscle protein mass, and the turnover of actin and myosin is ~1% per day (15).

Extracellular protein turnover represents only a small fraction of total body protein turnover. Albumin constitutes the largest mass of extracellular proteins, but its turnover is only ~0.15 g kg^{-1} day^{-1} (16) (similar to that of hemoglobin). Thus, cellular protein turnover represents the vast bulk of whole organism protein turnover.

Cellular proteins are also in a dynamic state. All intracellular proteins are degraded continuously, with half-lives varying more than 1000-fold (from a few minutes to >60 h) for individual protein species within the same cell (17). The mechanisms that underlie this remarkable specificity are only beginning to be elucidated. Variations in the activity of the machinery of protein degradation may involve modifications in the levels and activities of proteolytic enzymes (e.g., regulated by hormones, covalent modification, or interactions with inhibitors/metabolites, or by the trafficking/delivery of substrates to the loci for proteolysis).

Three major intracellular proteolytic systems are currently known. (*a*) Degradation of extracellular (and some membrane) proteins occurs within the cell's protease-rich vacuolar system (endosome/lysosome). Strictly speaking, this system for protein degradation is within the cell but is extracytoplasmic (i.e., the proteins targeted for degradation enter the cell via endocytosis and are degraded within the vacuolar lumen) (18–20). (*b*) The process of autophagy (21) involves membranous outgrowth from the endoplasmic reticulum (ER) and the nonselective engulfment and degradation of bulk cellular volume and its constituents. This process, which involves the lysosome and its constituent proteases, accounts for only a small amount of protein degradation under basal conditions but is markedly upregulated during starvation (22). Importantly, lysosomal targeting of extracellular proteins is dependent on oligo/monoubiquitination of receptor and other membrane proteins involved in routing the vesicles to the lysosome (23), whereas autophagy is dependent on signal-induced modification of a variety of proteins along the autophagic pathway by the autophagy (ATG) system, which is homologous to the ubiquitin system (24). (*c*) The ubiquitin

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proteolytic pathway is responsible for most cellular protein metabolism and provides both selectivity and specificity.

E1: ubiquitin-activating enzyme

E2: ubiquitin-conjugating enzyme

E3: ubiquitin-protein ligase

THE UBIQUITIN PROTEOLYTIC PATHWAY

The ubiquitin proteolytic pathway plays a crucial role in the degradation of most cellular proteins, including short-lived, regulatory, and misfolded/denatured proteins, and is therefore important in a variety of basic cellular processes. Among these are regulation of cell cycle and division, involvement in the cellular response to stress and extracellular modulators, modulation of cell surface receptors, and DNA repair (7, 25–27). The molecular mechanisms underlying these complex processes are poorly understood and many of the target proteins are unknown. In many cases ubiquitin modification serves nonproteolytic functions. The best-studied modification involves proteolysis of the target substrate and occurs in two discrete steps: (*a*) covalent attachment of multiple ubiquitin molecules to the protein substrate, and (*b*) degradation of the targeted protein by the 26S proteasome complex with recycling of free and reusable ubiquitin.

The ubiquitin pathway (**Figure 1**) (25, 26, 28, 29) consists of several components that act in concert. Conjugation of ubiquitin, a highly conserved 76–amino acid residue polypeptide, to the protein substrate proceeds via a three-step cascade mechanism. Initially, the ubiquitin-activating enzyme, E1, activates the C-terminal Gly residue of ubiquitin to a high-energy thiol ester with an internal E1 Cys residue. One of several E2 enzymes (ubiquitin-carrier proteins or ubiquitin-conjugating enzymes, Ubc's) transfers the activated ubiquitin, via an E2~ubiquitin thiol ester intermediate, to the substrate that is specifically bound to a member of the ubiquitin-protein ligase, E3, family. Transfer can be either directly to the E3 bound substrate, or via an additional E3~ubiquitin high-energy thiol ester intermediate. E3s catalyze the last step in the conjugation process, which is covalent attachment of ubiquitin to the substrate.

In most cases, the first moiety is transferred to an ε-NH_2 group of an internal Lys residue of the target protein. However, there are at least a dozen target proteins (e.g., MyoD, $p16^{INK4a}$, and the Human papillomavirus (HPV) oncoprotein E7 from strain 58) in which the first ubiquitination reaction involves fusion of ubiquitin to the free and exposed N-terminal residue of the substrate to generate a linear peptide bond (30). In successive reactions, a polyubiquitin chain is synthesized by processive transfer of additional activated ubiquitin moieties to an internal Lys residue of the previously conjugated ubiquitin molecule. It is important to emphasize that N-terminal ubiquitination is a novel pathway, distinct from the N-end rule pathway (31). In the latter, the N-terminal residue serves as a recognition and binding motif for the E3; however, ubiquitination occurs on an internal Lys residue(s) of the substrate. In N-terminal ubiquitination, modification occurs on the N-terminal residue, whereas recognition likely involves a downstream element(s).

The polyubiquitin chain serves as a recognition marker for the 26S proteasome. The structure of the ubiquitin system appears to be hierarchical: A single E1 appears to activate ubiquitin required for all modifications. It can transfer ubiquitin to several species of E2 enzymes. These enzymes, of which more than 20 were discovered in the human genome, act in concert with E3s. Each E2 enzyme can transfer ubiquitin to one or, more typically, several E3 proteins. Based on commonly shared structural motifs, it appears the human genome contains ~1000 E3s, although only a few have been characterized and their substrates identified. Two major families of E3 ligases have been described: the HECT (homologous to E6-AP C terminus) domain-containing E3s and the RING-finger domain-containing E3s, a more complex family in which the RING-finger protein is a common component but recognition is mediated by a different protein, such as an F-box

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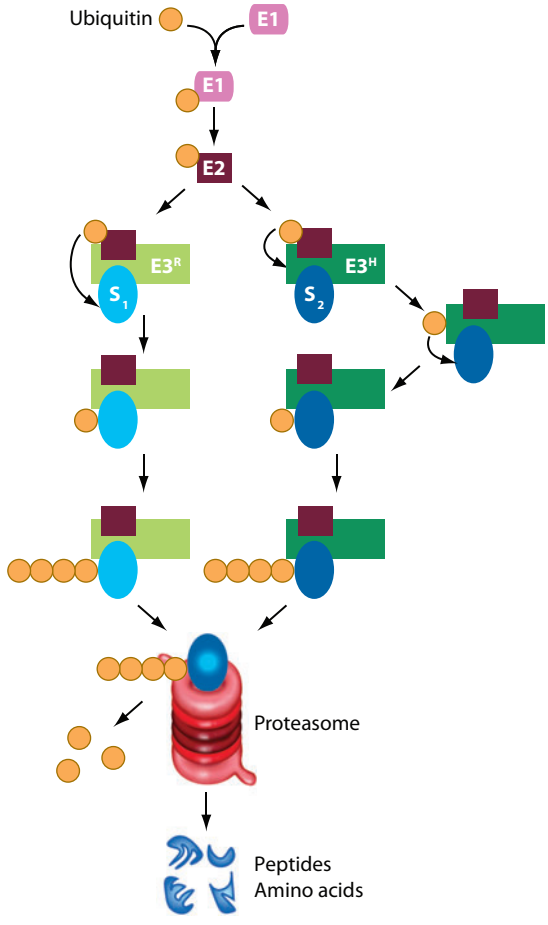

Figure 1

Sequence of events in the degradation of a protein via the ubiquitin-proteasome pathway. Activation of ubiquitin by the ubiquitin-activating enzyme, E1. Transfer of the activated ubiquitin from E1 to a ubiquitin-carrier protein, E2. Conjugation of ubiquitin mediated by RING-finger ubiquitin ligases ($E3^R$). Formation of a ternary complex between ubiquitin-charged E2, $E3^R$, and the target substrate (S_1), and initiation of synthesis of substrate-bound polyubiquitin chain by direct transfer of the activated ubiquitin moiety from E2 to the substrate. The $E3^R$ serves as a scaffold protein to which the substrate and the ubiquitin-charged E2 are bound, which enables the transfer of the activated ubiquitin moiety from E2 to the substrate. Formation of substrate-bound polyubiquitin chain by the successive conjugation of additional ubiquitin moieties to one another. Binding of the polyubiquitinated substrate to the 26S proteasome. Degradation of the ubiquitinated substrate to peptides. Conjugation of ubiquitin mediated by HECT domain ubiquitin ligases, $E3^H$. Generation of binary complex between the ubiquitin-charged E2 and the $E3^H$, and transfer of activated ubiquitin to the ligase. Transfer of the first ubiquitin moiety from $E3^H$ to the substrate (S_2). HECT domain E3s serve as catalysts to which the activated ubiquitin is first transferred from an E2. The E3s transfer it further on to the substrate and then to previously added ubiquitin moieties to generate the substrate-anchored polyubiquitin chain. Generation of substrate-bound polyubiquitin chain. Remainder of the pathway is as described above.

protein in the SCF (Skp2, Cullin, F-box) ubiquitin ligase family (e.g., pVHL, cullin, elongin B/C, or $SCF^{\beta TrCP}$) (26, 32). Other small families are the U-box and PHD domain-containing E3s. The E3 enzymes play a central role in the ubiquitin proteolytic pathway because they serve as the substrate-binding elements that endow the system with the high degree of specificity and selectivity it has toward its many cellular substrates. Because of their central role, their mechanism of action and modes of substrate recognition are of broad biological as well as clinical importance (33).

Following conjugation, the protein moiety of the adduct is degraded, and free and reusable ubiquitin is released via the activity of deubiquitinating enzymes (DUBs). A multisubunit 26S proteasome complex that specifically degrades conjugated proteins in an ATP-dependent manner has been purified and characterized (4, 6, 34, 35). The core catalytic unit of the protease is a ~700 kDa 20S proteasome complex. Two 19S cap complexes associate with the 20S cylindrical structure to generate a 26S dumbbell-shaped complex. These 19S complexes are regulatory subunits that impart specificity and control (36). The 26S proteasome also contains one or more ubiquitin C-terminal hydrolases (isopeptidases) involved in the recycling of ubiquitin. Little is known of the regulation of the 26S protease complex (37).

An important yet unresolved problem involves the conversion of a cellular protein into a substrate. For some proteins, the process is constitutive, i.e., they are recognized without modification. For others, recognition requires association with ancillary proteins or a posttranslational modification such as phosphorylation, oxidation, acetylation, etc. Experimental evidence indicates that in addition to the three classical conjugating enzymes (E1, E2, E3) and the 26S proteasome, ancillary proteins are required for the conjugation or degradation of certain specific protein substrates.

Until recently, the ubiquitin system was viewed as a complex molecular pathway for proteolysis, limited to cytoplasmic, nuclear, and some membrane proteins, and dependent on internal lysine residues and a myriad of E3 ligases, as described above. It is now clear, however, that ubiquitination also serves as a signal for trafficking, kinase activation, and other nonproteolytic functions (3, 27, 38).

A number of distinct ubiquitin-like proteins, including SUMO, Nedd8, and Atg8, have been found to also function as protein modifiers in a multitude of cellular processes from signal transduction to enzyme activation, regulation of autophagy, and cell cycle control. Dysregulation in their function has implications for the pathogenesis of several human diseases (1).

We discuss below five human pathophysiological states in which the ubiquitin proteolytic pathway plays a central role: response to hypoxia; inflammatory diseases; neurodegenerative diseases; muscle-wasting disorders; and certain malignancies, including human papillomavirus–induced uterine cervical carcinoma.

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RESPONSE TO HYPOXIA

To adapt to different oxygen tensions in different tissues multicellular organisms have developed a variety of sensors that respond to the changing oxygen tensions and allow optimal delivery and utilization of oxygen. Hypoxia-inducible factor 1 (HIF-1) is a key mediator of the adaptive response to reduced oxygen availability in multiple pathophysiological states. HIF-1 mediates these effects via transcriptional regulation of genes that encode proteins essential for tissue oxygen delivery and energy homeostasis, including those involved in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation and survival (39).

HIF-1 was discovered by identifying a hypoxic response element in the enhancer of erythropoietin (EPO), a hormone that stimulates erythrocyte proliferation and undergoes hypoxia-induced

transcription (40, 41). HIF-1 is a basic helix-loop-helix heterodimer comprised of the hypoxia-induced subunit HIF-1α and the constitutively expressed subunit HIF-1β, also known as aryl hydrocarbon receptor. HIF-1β's protein level is maintained independent of oxygen availability. HIF-1α, in contrast, is tightly regulated by oxygen: It is rapidly degraded (t1/2 < 10 min) under normoxic conditions, but under hypoxic conditions it becomes stable. It binds HIF-1β, and the complex is translocated from the cytoplasm to the nucleus where it becomes transcriptionally active following recruitment of coactivators such as p300/CBP. This stimulates the transcription of a large group of proteins, such as vascular endothelial cell growth factor (VEGF), EPO, transferrin, and their corresponding receptors, and ferritin, all of which utilize oxygen efficiently under those conditions. HIF-1α's transcription and synthesis are unaffected by oxygen. Thus, the key event in oxygen sensing, regulation of HIF-1, is mediated via protein degradation (42–44).

Oxygen-dependent regulation of HIF-1α protein degradation is mediated by a functional domain of 200 amino acids termed the oxygen-dependent degradation domain. Within this domain of HIF-1α, prolyl hydroxylases utilize oxygen to generate 4-hydroxyproline at residues 402 and/or 564 in the human molecule (45, 46). This hydroxyproline-modified HIF-1α serves as a substrate for ubiquitin-proteasome-mediated degradation (**Figure 2**).

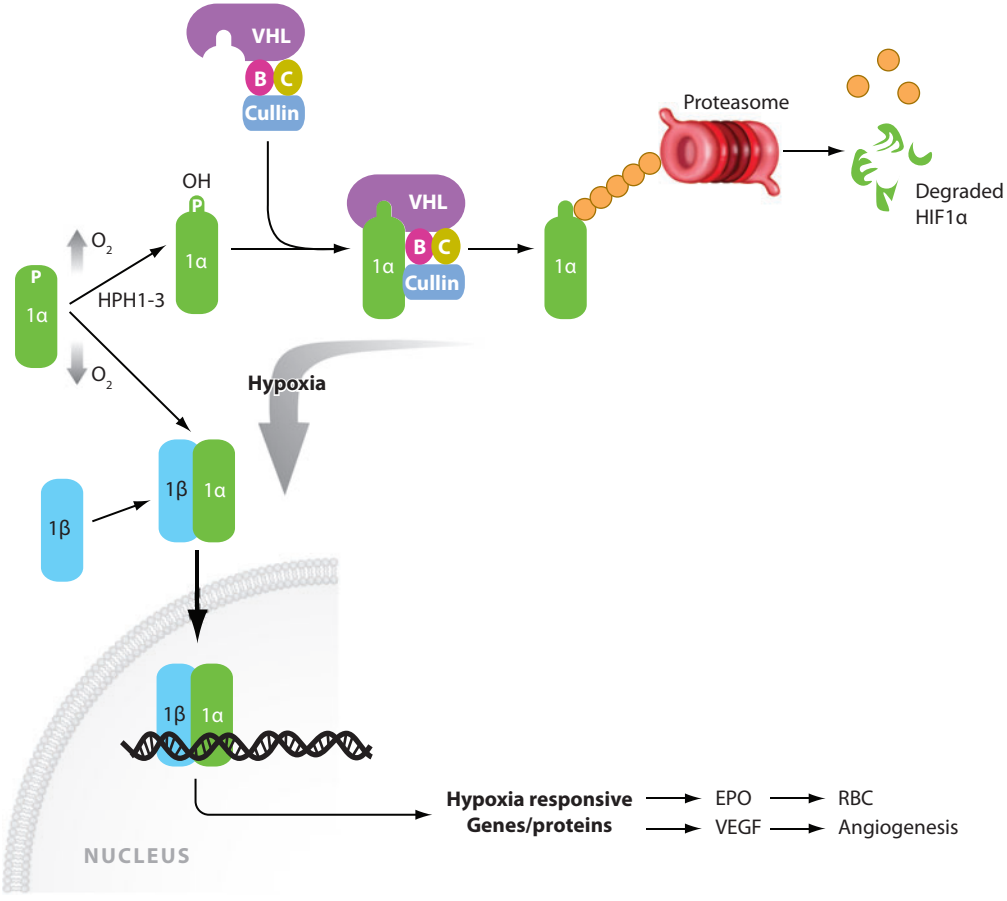

Figure 2

Ubiquitin-proteasome-mediated degradation of hypoxia-induced factor (HIF) 1.

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Under normoxic conditions, HIF-1α is hydroxylated on a proline (P) residue via HPH1-3. The hydroxy-proline HIF-1α is recognized by the ubiquitin E3 ligase complex (VHL, elongin B/C, cullin), polyubiquitinated, and rapidly degraded by the proteasome. Under hypoxic conditions, hydroxylation of HIF-1α does not occur and HIF-1α forms a heterodimer with its HIF-1β partner, is translocated to the cell nucleus, and activates the genetic program for the hypoxic response (e.g., inducing EPO and VEGF). Three such prolyl hydroxylases have been identified in mammalian cells. Each displays distinct tissue distribution, subcellular localization, and regulation by both hypoxia and ubiquitin-mediated degradation. This suggests that these enzymes may modulate a tissue-specific hypoxia response via multiple mechanisms. Interaction of the prolyl hydroxylases and HIF-1α is enhanced by a binding partner, OS-9, a protein of otherwise unknown function, further promoting HIF-1α ubiquitination and degradation.

Hydroxylated HIF-1α serves as a substrate for the ubiquitin ligase complex, pVHL/elongin B, C/cullin-2/Rbx1, which polyubiquinates HIF-1α and presents it to the proteasome for degradation. The hydroxylated prolines constitute a recognition signal for the von Hipple-Lindau (VHL) tumor suppressor. Within the complex, the pVHL protein binds to elongin-C, which recruits elongin-B, cullin-2, and Rbx1 (to form the BCV-Cul2 E3 ligase complex) (47) (**Figure 2**). Structural studies demonstrate a binding pocket within pVHL for the hydroxylated proline(s).

pVHL is a tumor suppressor mutated in virtually all renal cell carcinomas and in some other vascularized tumors. In cells/tissues deficient in functional VHL, HIF-1α is stabilized and active under normoxia (48). The VHL E3 ligase complex is ubiquitously expressed and generally localized to the cytoplasm; its shuttling to the nucleus allows HIF-1α degradation in both of these subcellular compartments.

Semenza (49) recently reported a new HIF-1α-interacting protein, receptor for activated C-kinase 1 (RACK1), which can promote HIF-1α degradation independent of oxygen, prolyl hydroxylase, and VHL. RACK1 recruits HIF-1α to the elongin B/C subunits of the E3 ligase complex independent of VHL, whereby HIF-1α is ubiquitinated and then degraded via the proteasome. Thus, RACK1 appears to serve as a counterpart of VHL in an oxygen-independent HIF-1α degradation pathway. As basal levels of HIF-1α vary among tissues and cell types, RACK1 may well serve to regulate basal HIF-1α levels, whereas prolyl hydroxylases mediate oxygen-induced changes in HIF-1α (50).

A wide variety of target genes and pathways of HIF-1 are mediated via its degradation. Indeed, estimates in endothelial cells suggest that >2% of all human genes may be HIF-1 regulated (51). More than 60 genes have already been described as direct targets of HIF-1 (52). Two examples of important pathways regulated by HIF-1 are erythropoiesis and angiogenesis (53).

Hypoxia increases the expression of erythropoietin, which enhances red blood cell production and thus oxygen delivery to tissues. As increased red blood cell production depends on sufficient ferric iron (hypoxia upregulates the iron-transport protein transferrin and its receptor, which mediates cellular iron uptake) and ceruloplasmin, which serves as a ferroxidase to provide ferric from ferrous iron.

Angiogenesis is a complex process involving multiple cell types, programs, and genes, many of which are induced by hypoxia (54). Among these, the most potent endothelial mitogen is VEGF. Herein, an increase in vascular density results in a decrease in oxygen diffusion distance. HIF-1α and its hypoxic response regulate VEGF expression as well as genes that govern vascular tone, such as nitric oxide synthase, endothelin 1, etc.

Thus, not surprisingly, activation of HIF activity has been seen in a wide array of physiological responses to ischemia and hypoxia. For example, levels of HIF-1α and VEGF increase in myocardium following acute coronary occlusion (55), in the pre-eclamptic placenta (56, 57), and in the ischemic retina (58).

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The therapeutic opportunities inherent in understanding hypoxia signaling in pathophysiological states are myriad, complex (e.g., different in whole organisms than in isolated cells), and in their infancy. Pharmacological interventions are being aimed at hypoxic or ischemic conditions as well as malignancy. The potential targets include HIF, its E3 ubiquitin ligase complex, and the three HIF prolyl hydroxylases. Mimetics of the HIF-1α oxygen-dependent degradation domain and the resultant indirect effects on HIF-2α (59) represent direct HIF-1 therapeutics. HIF prolyl hydroxylase inhibitors activate HIF target gene programs, including those of angiogenesis and erythropoiesis, yet the pliotrophic nature of the HIF response is complex (60). A variety of anticancer agents inhibit HIF-1 activity by decreasing the rate of HIF-1α synthesis, increasing the rate of degradation, or both. Other HIF inhibitors interfere with the activation of transcription of HIF-1 target genes (52). Thus, a number of novel anticancer agents are now being recognized as modulators of HIF-1 biology via a host of molecular mechanisms.

INFLAMMATORY DISEASES

Inflammatory diseases result from physiological and pathophysiological modulation of the innate and adaptive immune responses. The initiation and propagation of these responses are mediated by a wide variety of genes, many of which are controlled by the transcription factor, nuclear factor-κB (NF-κB) (61, 62). NF-κB is activated upon engagement of cell surface toll-like receptors (TLRs) on immune cells by pathogen-associated molecular patterns (PAMPs) from bacteria, viruses, fungi, or parasites. The activation of NF-κB results in a cell-specific genetic response, including elaboration of proinflammatory cytokines (e.g., tumor necrosis factor, interleukin 1β) that generate a positive autoregulatory feedback loop resulting in expression of adhesion molecules, chemokines, etc., which enhance the inflammatory response. Uncontrolled or overexpressed NF-κB activation underlies many inflammatory states, including septic shock, arthritis, asthma, ischemia-reperfusion injury, etc.

The ubiquitin system is involved in at least three significant but completely distinct roles in the NF-κB pathway (**Figure 3**) (63, 64). These roles appear to modulate the intensity, timing, and diversity of the inflammatory response.

1. NF-κB is a heterodimeric transcription factor composed of two Rel family members. The prototype and most prevalent dimer is p50/p65. p65 is constitutively synthesized and the protein is localized to the cytoplasm. p50, in contrast, is synthesized as a larger, inactive precursor, p105, which is specifically degraded via the ubiquitin-proteasome system to generate the mature active product p50 (**Figure 3*a***). This selective degradation is precisely regulated. Indeed, this processing represents a well-characterized example for limited degradation of a substrate protein via the ubiquitin-proteasome system (65). p50 is derived from the

Figure 3

Roles of the ubiquitin-proteasome system in the NF-κB pathway of inflammatory response. (*a*) p50 is generated by the ubiquitin-proteasome system following cleavage and removal of the C-terminal domain of the p105 precursor. p50 combines with p65 forming the p50/p65 NF-κB dimer, which is maintained in an inactive state in the cytosol via complex with IκB. (*b*) Extracellular cytokine TNF binds to the membrane TNF receptor, which complexes with intracellular signaling/regulatory molecules TRADD, RIP, and TRAF2, which is polyubiquitinated on Lys 63 of ubiquitin. A deubiquitinating enzyme, CYLD, removes ubiquitin from TRAF2 and recycles unconjugated TRAF2. RIP is polyubiquitinated on Lys 48, which targets it to proteasomal degradation, and on Lys 63, which promotes signaling with TRAF2 and TRADD. Dissociation of the trimeric complex initiates RIP Lys 63 polyubiquitination, which is recognized by the IKK (inhibitor of κB kinase) trimeric complex, and promotes dual phosphorylation of IκB. (*c*) The dual phosphorylated IκB is then polyubiquitinated and degraded via the proteasome, releasing the p50/p65 complex, which enters the nucleus via the unmasked NLS and promotes the NF-κB responsive genetic program.

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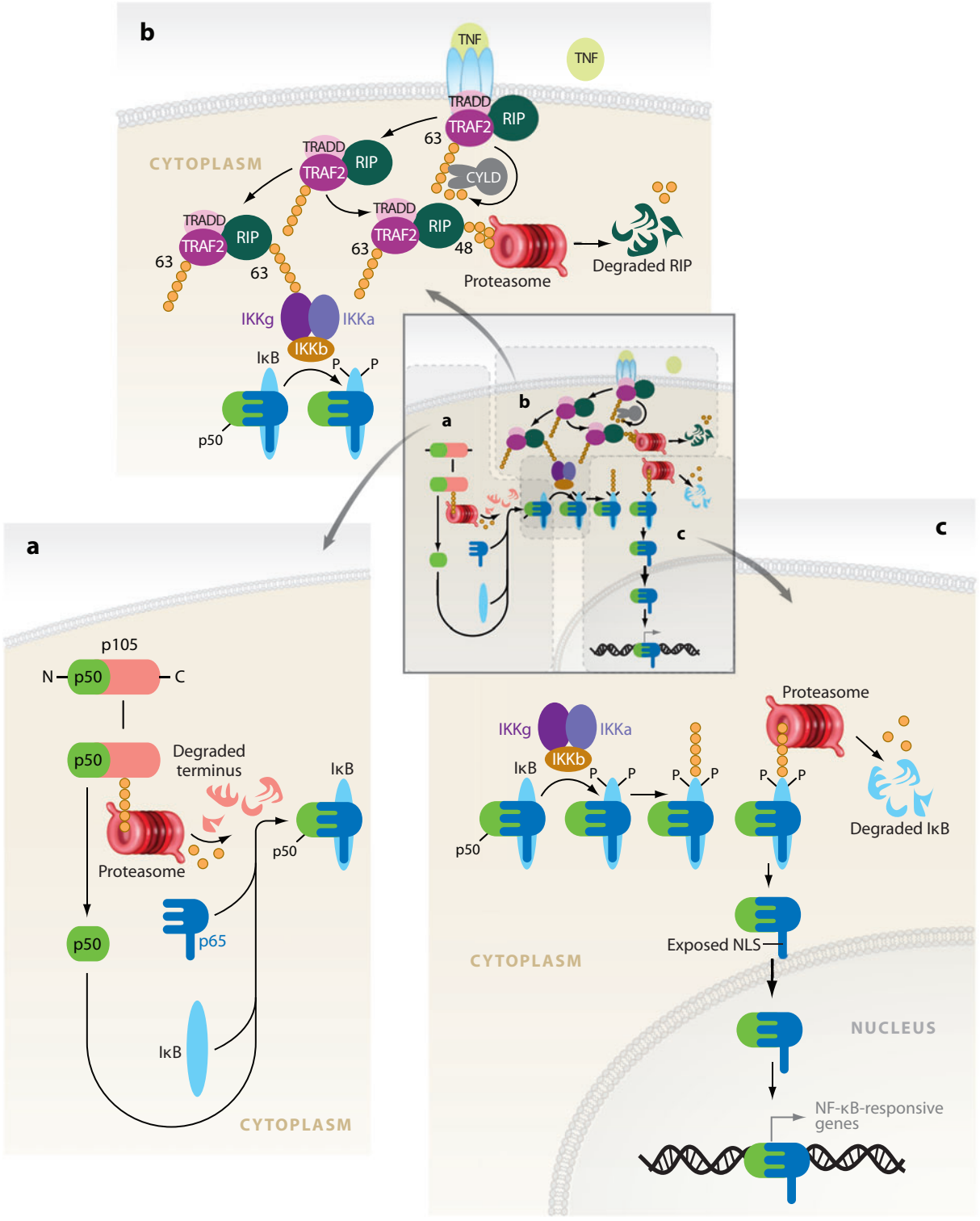

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N-terminal domain of p105, whereas the C-terminal domain is degraded. A glycine-rich region within the C-terminal domain is a key processing signal because it interferes with the proteasome's ability to completely destroy the ubiquitinated p105 precursor. Additional sites internal to p105 are required for the precise processing of p105 to p50 (66, 67) (**Figure 3*a***).

2. The ubiquitin-proteasome pathway is involved in the generation of the IKK (inhibitor of κB kinase)-activating complex. This complex regulates the association of IκB (inhibitor of κB) with the p50/p65 complex. IKK is generated following engagement of cell surface receptors (**Figure 3*b***). For example, tumor necrosis factor (TNF) binding to TNF receptor leads to the recruitment of TNF receptor-1-associated death domain protein (TRADD), which then recruits TNF receptor-associated factor 2 (TRAF2) and thereafter the receptor interacting protein (RIP). This quaternary complex activates the E3 ligase activity of TRAF2. Activated TRAF2 catalyzes its own polyubiquitination, release into the cytoplasm, and subsequent polyubiquitination of RIP. Ubiquitinated RIP is recognized by the IKK trimeric complex and promotes the phosphorylation and thus activation of IKK, now able to phosphorylate IκB (**Figure 3*b***) (68, 69). Interestingly, TRAF2 is polyubiquitinated via ubiquitin's internal Lys 63 linkages. RIP is polyubiquitinated via ubiquitin's internal Lys 63 to promote signaling, as well as via Lys 48 linkages, which target RIP to proteasomal degradation. In addition, a deubiquitinating enzyme, cylindromatosis tumor suppressor protein (CYLD), acts on the TRAF2 polyubiquitin chain to release ubiquitin and recycle TRAF2. Similar to its role in TNF receptor signaling, the ubiquitination of TRAF6, a mediator of IL-1 receptor action, also promotes the phosphorylation and activation of IKK.
3. The p50/p65 heterodimer is sequestered in the cytoplasm by the binding of a member of the IκB family, most often IκBα. The inhibitor masks the nuclear localization sequence of p65, thus restricting the p50/p65 complex from entry to the nucleus. The cytoplasmic retention of the inhibited complex (p50/p65/IκB) is released upon kinase-mediated dual phosphorylation of IκB (**Figure 3*c***). This diphosphorylated form of $I\kappa B^{Pser32,36}$ is rapidly recognized by the ubiquitin ligase, $SCF^{\beta TrCP}$. Upon recognition, IκB is polyubiquitinated and thereafter degraded by the proteasome. The kinase responsible for the dual phosphorylation of IκB and thus generation of the E3 ligase substrate is a trimeric complex (IKKα, IKKβ, and IKKγ) described above (2). Upon IκB degradation, the p50/p65 complex is able to translocate into the nucleus and initiate the inflammatory gene response program.

Therefore, there are currently at least three distinct roles for the ubiquitin system in NF-κB signaling: (*a*) C-terminal limited processing of p105 to generate p50; (*b*) polyubiquitination of intermediates in TNF- and IL-1 receptor signaling (RIP and TRAF6) pathways, which promote IKK activation; and (*c*) phosphorylation-dependent degradation of IκB, which releases active p50/p65.

Disease-based implications of these pathways have recently evolved. NF-κB mediates generation of multiple critical cytokines in inflammatory arthritis. In the peptoglycan/polysaccharide-induced inflammatory arthritis model, inhibition of proteasomal activity was associated with decreased NF-κB activation, reduction of cytokine and cell adhesion molecules, and a decrease in arthritis score. Similarly, in a streptococcal cell wall model of arthritis, proteasomal inhibition administered either prophylactically or as treatment during the course of disease was successful in reducing inflammatory mediators as well as the clinical course of disease, including inflammation and cartilage and bone destruction (70).

Asthma represents another inflammatory state in which numerous mediators are dependent on NF-κB activation. In an animal model of pulmonary immune sensitivity and hypereosinophilia, intratracheal administration of proteasome inhibitor resulted in reduction in pulmonary eosinophils

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and inflammation (71). Further, there was a synergistic response to a low dose of proteasome inhibitor supplemented with a low dose of a glucocorticoid.

Inflammation is a prominent feature of ischemia-reperfusion injury in both the heart and brain. In rodent models of cerebral ischemia-reperfusion inflammatory injury, proteasomal inhibition reduced both inflammatory mediators and limited brain injury (72). Further, proteasome inhibitors offered neuroprotection even when administered several hours following the cerebral reperfusion (73, 74). In models of cardiac ischemia-reperfusion injury, proteasomal inhibition was associated with reduction in NF-κB activation, inflammatory cell infiltration, infarct size, and an increase in survival (75, 76). Finally, it is becoming more apparent that there is an intimate interrelationship among inflammation, immunity, and malignancy. The role of soluble mediators (e.g., cytokines) in tumor initiation, promotion, and progression is being dissected mechanistically. Herein, NF-κB has emerged as one critical link (77). Modulators of the NF-κB pathway are attractive potential therapeutic agents. To date, both natural and designed molecules have been studied at virtually every level of the pathway (78). In general, these fall into three categories: interruption of extra- and intracellular signaling; modulation of the steps of NF-κB activation/degradation; and modulation of NF-κB nuclear activity, including nuclear translocation and DNA binding/transcriptional machinery interactions. For example, antioxidants, cyclosporin, and aspirin interfere with early steps in cellular signaling prior to the IKK complex. Proteasome inhibitors, protease inhibitors, and cyclosporin affect IκB degradation; whereas within the nucleus, glucocorticoids affect both transactivation and IκB upregulation. Specifically, with regard to the roles of the ubiquitin system and the NF-κB pathway, several areas are active in therapeutic development. These include proteasome (e.g., lactacystine, boronic acid peptide, and ubiquitin ligase inhibitors) and protease inhibitors (e.g., N-benzoyl-L-tyrosine-ethylester) that inhibit NF-κB. In addition, a wide variety of molecules (e.g., nitric oxide, ibuprophen, and sulindac) inhibit IκB phosphorylation.

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NEURODEGENERATIVE DISEASES

A growing body of evidence supports the association of several chronic neurodegenerative diseases with the presence of inclusion bodies associated with ubiquitinated proteins. These include the neurofibrillary tangles of Alzheimer's disease, the brainstem Lewy bodies of Parkinson's disease, and the nuclear inclusions of CAG repeat disorders such as Huntington's disease and spinocerebellar ataxias. Whereas direct linkage of the pathophysiology to the ubiquitin system is clear in some instances, it is not yet evident in others. This is further complicated by the notion that previously these protein aggregates were considered pathological, whereas more recently they are viewed as a potential protective mechanism to spare cellular machinery from the toxic effects of the soluble proteins (see 79–82) (**Figure 4**).

Alzheimer's disease, identified by progressive (especially short-term) memory loss, is characterized histopathologically by two forms of protein deposits in the brain: extracellular amyloid plaques and intraneuronal neurofibrillary tangles (83). The plaques are rich in amyloid β peptides (Aβ) generated via proteolytic cleavage of the transmembrane amyloid precursor peptide (APP) by a series of secretases resulting in $A\beta^{1-40}$ and $A\beta^{1-42}$ that misfold extracellularly and form fibrils and plaques. The neurofibrillary tangles are rich in tau, a microtubule-associated protein, and its hyperphosphorylated forms.

Four general lines of evidence suggest linkage of the ubiquitin-proteasome system to Alzheimer's disease pathogenesis: (*a*) the biology of an unusual variant of ubiquitin, termed UBB^{+1}; (*b*) aberrant proteasomal activity; (*c*) mutated ubiquitin hydrolyases; and (*d*) related activities of certain ubiquitin ligases.

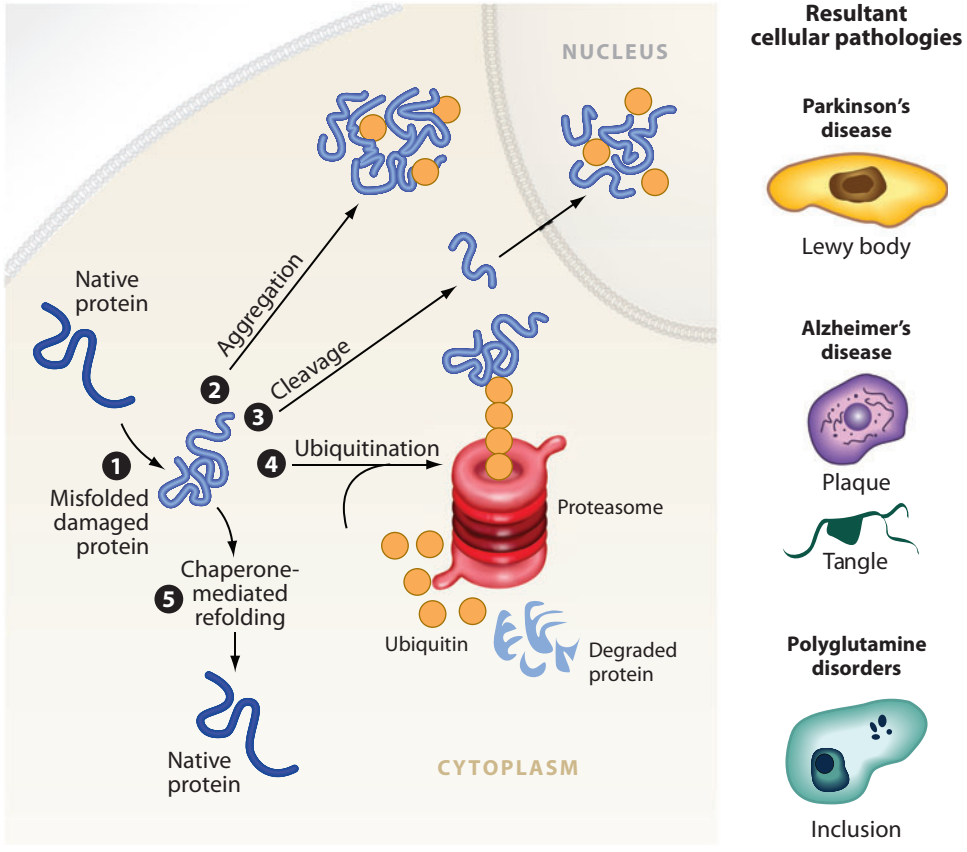

Figure 4

Ubiquitin-proteasome system and pathogenesis of neurodegeneration. Three paradigms of neurodegeneration (Parkinson's disease, Alzheimer's disease, and polyglutamine disorders) are schematized herein and discussed in the text. A variety of triggers, both endogenous (e.g., mutation) and exogenous (e.g., oxidative stress) generate (1) misfolded proteins, which undergo several potential fates: (2) aggregation, (3) cleavage, (4) ubiquitination and proteasomal degradation, or (5) chaperone-mediated refolding. Ubiquitinated misfolded protein or fragments thereof may accumulate in the nucleus or cytoplasm or be degraded via the proteasome. The resultant cellular pathologies are seen in the right panel: Lewy bodies in PD, plaques and tangles of AD, and intracellular inclusions of PolyQ diseases. Adapted from (79).

UBB^{+1} is a genetic variant of ubiquitin with a 19–amino acid C-terminal extension, first identified in Alzheimer's disease neurons and subsequently found in neurons of other neurodegenerative disorders (84). The C-terminal extension renders UBB^{+1} unable to covalently tag protein substrates, as the terminal ArgGlyGly of ubiquitin is not free (see above, **Figure 1**). However, UBB^{+1} can be ubiquitinated via its internal lysine residues and thus may serve as a dominant negative substrate for the proteasome (85). Further, ubiquitinated UBB^{+1} is not effectively deubiquitinated. Whereas UBB^{+1} at low concentrations serves as a proteasomal substrate, at high concentrations it inhibits proteasomal function (86). The mechanisms underlying UBB^{+1}-mediated neuronal degeneration appear to involve neuritic beading and mitochondrial stress (87), suggesting development of potential neuroprotective strategies via interference with UBB^{+1} and its biology.

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Proteasomal activity has been the subject of recent focus in Alzheimer's disease pathogenesis. Researchers have seen region-specific reduction in proteasomal activity in Alzheimer's disease brain. Herein, regions most abnormal histopathologically (e.g., hippocampus) are those with the most reduced proteasomal activity (88, 89). Whether the reduction in proteasomal activity is causal or resultant from neurodegeneration (or aging) is not yet clear. However, in vitro Aβ reduces proteasomal activity (90), suggesting increased Aβ may in part underlie diminished proteasomal activity in Alzheimer's disease.

Recently, a new member of the ubiquitin hydrolase family, UCH-L1, has been implicated in synaptic dysfunction and memory loss. Enhanced UCH-L1 expression (to supplement endogenous UCH-L1) had a protective effect on memory loss in a mouse model of Aβ accumulation and Alzheimer's disease (91). UCH-L1 is distinct from its homolog UCH-L3 in terms of tissue localization, enzymatic activity, and relationship to ubiquitin proteasomal function. It is possible that UCH-L1 is involved in several protein aggregation-dependent neurodegenerative diseases and not specific to Alzheimer's disease. One potential mechanism could involve the lack of released ubiquitin and the generation of its local deficiency for other cellular functions. Regardless, its mechanism of action and mode of regulation will be important avenues to define.

The cleavage of APP to Aβ requires the action of presenilins, components of the multiprotein γ secretases. The presenilins are degraded via the ubiquitin-proteasome system in a process mediated by an E3 SCF complex containing the F-box protein FBXW7 (92, 93). In addition, humanin, a neuroprotective 24–amino acid peptide, appears to suppress presenilin-mediated Aβ neurotoxicity in Alzheimer's disease and is degraded via the ubiquitin-proteasome system with the RING protein TRIM serving as its E3 ligase (94).

Although many of these activities may be engaged in the pathogenesis of Alzheimer's disease, it is likely that they play roles in other protein aggregation and neurodegenerative disorders as described below. Parkinson's disease is characterized by the progressive loss of neurons in the substantia nigra and loss of dopaminergic innervation in the striatum, with residual neurons expressing cytoplasmic Lewy body inclusions. Although there is not a single central role that can be attributed to an aberration in the ubiquitin-proteasome system that may underlie Parkinson's disease pathogenesis, several recent findings support a variety of interactions. One of the most intriguing is that of Parkin, a 52kDa E3 ubiquitin ligase with two RING-finger motifs. Point mutations and deletions in the Parkin gene have been found in ~50% of patients with autosomal recessive early onset Parkinson's disease, one of the most common familial forms (95, 96). Although wild-type (WT) Parkin appears to bind to the proteasome via its S5a subunit, thus facilitating ubiquitinated conjugate delivery, mutations at R42 result in reduced S5a binding (97). Further, Parkin is reported to be a component of the E3 SCF complex containing F-box/WD repeat proteins analogous to the VHL complex discussed above. Many mutations that reside within the RING fingers have been described, and not all result in loss of activity (98, 99). West et al. (100) suggest that haploinsufficiency of Parkin can also be associated with Parkinson's disease. However, the development of a Parkin-null mouse has not clarified the role of Parkin in this pathogenesis (101).

To date, focus on the Parkin substrates has not clarified their role in Parkinson's disease pathobiology (102). Imai and colleagues (99) and others proposed that defective Parkin action, which results in accumulation of its substrates, can result in toxicity to dopaminergic neurons. Lack of ubiquitination that serves a nonproteolytic function, such as activation of the target protein, may also underlie the observed pathology. Although several Parkin substrates are now known, their linkage to dopaminergic neurotoxicity is not established. For example, Parkin-associated endothelial-like (Pael) receptor serves as a Parkin substrate (99). When overexpressed, Pael receptor aggregates upon misfolding and initiates the unfolded protein response, a stress response

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of the endoplasmic reticulum. Parkin ubiquitinates and promotes degradation of the insoluble Pael receptor in concert with two endoplasmic reticulum–associated E2s. Importantly, insoluble Pael receptor accumulates in autosomal recessive Parkinson's disease brains harboring mutant Parkin. Perhaps most striking is that Parkin can protect dopaminergic cells in *Drosophila* from neurotoxicity induced by overexpressed Pael receptor (103).

A second Parkin substrate of interest is the p38 subunit of aminoacyl tRNA synthetase complex (98), which when overexpressed results in Parkin-containing aggregates. Elimination of proteins containing an expanded polyglutamine stretch (see below) via Parkin is also of note; however, this may not be related to its role in the pathogenesis of Parkinson's disease.

Finally, a *Drosophila* Parkin-null mutant, which displays locomotor disorders and reduced life span, is dysfunctional in a subset of dopaminergic neurons (104). Precisely how this relates to the mammalian pathophysiology is not yet clear. Thus, the role(s) of Parkin as an E3 ubiquitin ligase, the ligase's substrates, and the number of mutants of Parkin associated with autosomal recessive Parkinson's disease that are enzymatically inactivating mutations will likely provide avenues for both better understanding the underlying pathogenetic mechanisms and development of possible therapeutic strategies.

CAG repeat disorders, including Huntington's disease and spinocerebellar ataxias, are characterized by a toxic gain of function of the disease-associated expanded polyglutamine (polyQ) protein, the development of ubiquitin-positive neuronal intracellular inclusions, and eventual cell death in a subset of neurons. Each of the nine known CAG repeat diseases displays an increased number of glutamine residues in the mutant protein, but they are distinct in both neuropathological and clinical presentations (105). Many patients display both clinical onset of symptoms in midlife and slow progression consistent with prolonged exposure/accumulation of the disease protein.

Huntington's disease is caused by a mutation in the gene encoding the huntingtin protein, which may be involved in gene transcription (106). For Huntington's disease and several of the other CAG repeat diseases there is strong evidence for a toxic gain of function. However, loss of function of huntingtin encoded by the WT allele has not been excluded because the mutant protein not only undergoes self aggregation, but it does so with WT protein (107). The CAG disorders exhibit amyloid-like fibrils of β-strands of the mutant proteins. These form aggregates in the cytoplasm and/or nucleus. Multiple proteins involved with the ubiquitin-proteasome system are associated with these aggregates and include ubiquitin, E2/E3s, and proteasome subunits. These intracellular inclusions may function to protect the cell via sequestration of the toxic misfolded proteins. For example, expression of a dominant negative form of the E2 Cdc34p decreased the intranuclear inclusions but increased cell death in a model of Huntington's disease (108).

WT, as well as mutant, CAG disease proteins interact with the ubiquitin system components. For example, huntingtin is ubiquitinated by 25-kDa E2. Further, an overexpressed mutant huntingtin inhibits proteasomal activity, which results in cell cycle arrest (109).

The polyglutamine stretch may inhibit access of the ubiquitinated proteins to the proteasome and thus may inhibit activity. In fact, glutamyl-lysine crosslinks in proteins resulting from transglutaminase action further inhibit proteasomal activity (110). An alternative explanation is that the proteasomal capacity is exceeded by the available misfolded substrates. Support for his hypothesis comes from inducible transgenic mouse studies (e.g., 111). The consequences of proteasomal inhibition within neurons certainly include the direct, toxic sequelae of proteins whose accumulation is not physiologically tolerated. Thus, although accumulation of CAG repeat proteins pose a direct neurotoxic challenge to cells, abrogation of the inhibition of the ubiquitin proteasomal system may offer therapeutic advantages.

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MUSCLE WASTING DISORDERS

Accelerated muscle proteolysis is the primary cause of the loss of lean (nonfat) body mass characteristic of many disease states, including fasting/starvation, uncontrolled diabetes mellitus, sepsis, cancer cachexia, renal failure, muscle disuse, and denervation, etc. These pathophysiological conditions result in preferential loss of protein from skeletal muscle with sparing of visceral organs and brain. There is often an accompanying decrease in protein synthesis. Although evidence suggests that calcium-dependent proteolysis, caspases, and lysosomal degradation contribute to accelerated muscle proteolysis, the major pathway responsible is the ubiquitin-proteasome system (112, 113).

The acceleration in muscle proteolysis that accompanies these disease states is rapid, leads to both loss of muscle mass and protein content, and is characterized by a common set of biochemical changes (112). Actin and myosin, the generally long-lived myofibrillar proteins that make up ~65% of muscle protein, are substantively degraded. Inhibitors of ATP production abrogate the accelerated proteolysis. Coincident with increase in the content of ubiquitinated proteins, enhanced proteolysis-specific responses include increase in expression of mRNA for ubiquitin, some E2s, E3s, and several subunits of the proteasome. This supports the notion of a coordinated physiological adaptation (114, 115). Furthermore, inhibition of the proteasome abrogates the accelerated proteolysis (116).

A distinct program of gene expression is initiated during skeletal muscle atrophy (117, 118). Differential expression screening studies designed to identify markers of the atrophic muscle by the Goldberg and Glass groups identified two E3 ubiquitin ligases, termed atrogin-1 (119) or MAFbx and MuRF1 (120). Thirteen models of muscle wasting and accelerated proteolysis have been shown to result in an increase in MAFbx/Atrogin and MuRF-1 (reviewed in 121). MAFbx/Atrogin is an F-box E3 and likely functions in an SCF complex, whereas MuRF1 is a RING-finger E3. Both are relatively specifically expressed in muscle. Mice null for MAFbx or for MuRF1 are phenotypically normal, however, under atrophying conditions and display less loss of muscle mass/protein than control littermates (120). Although the MAFbx and MuRF1 substrates are largely unknown, MuRF1 has been shown to bind titin, a myofibrillar M line protein (122). Further, both MAFbx and MuRF1 are upregulated during sepsis (123). Thus, MAFbx and MuRF1 may serve as potential therapeutic targets. It is likely other E3 ligases are involved in the pathobiology of muscle wasting, as well.

The molecular regulation of MAFbx and MuRF1 appears complex. Studies in cultured cells and in vivo have shown that atrophy-induced upregulation of MAFbx and MuRF1 can be antagonized by IGF1 via the PI3K/Akt pathway and the FOXO transcription factors (124, 125). This effect (i.e., activation of the Akt pathway) is also sufficient to block glucocorticoid-induced muscle atrophy (125).

The NF-κB signaling pathway (see above) is also involved in muscle atrophy. Activation of NF-κB in mice results in increased MuRF1 (but not MAFbx) expression and in skeletal muscle atrophy (126). This suggests differential regulation of the MuRF1 and MAFbx pathways.

Thus, accelerated muscle proteolysis occurs during a wide variety of pathological disease states via a common set of biochemical and transcriptional changes characteristic of an atrophy program. One common physiological mediator appears to be glucocorticoids, which are essential for many of the characteristic changes seen in several types of atrophy in vivo (115, 127). Glucocorticoids increase overall myofibrillar protein degradation and induce both MAFbx and MuRF1, as well as a host of other atrophy-associated genes (128). Physiologically, IGF1 and/or insulin appear to function as a dominant signal over glucocorticoids. Thus, maintaining high levels of insulin or IGF1 when glucocorticoids are in excess offers potential therapeutic benefit.

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HUMAN PAPILLOMAVIRUSES, CERVICAL CANCER, AND OTHER MALIGNANCIES

Papillomaviruses, with more than 130 distinct types, are small DNA viruses that infect epithelial cells and are associated with benign conditions (e.g., warts) and cervical carcinoma. The 30 HPV subtypes associated with the anogenital tract are classified as high or low risk. Among the high-risk HPVs, which carry a significantly increased risk of developing cervical cancer, the two most clearly associated with the development of this type of cancer are types 16 and 18. More than 95% of cervical carcinomas harbor at least one copy of a high-risk HPV genome.

The E6 and E7 genes are the only HPV genes generally expressed. Cell culture and animal studies have demonstrated that both E6 and E7 function as oncoproteins (129, 130). E6 interacts with the tumor suppressor protein p53 (131). E7 interacts with the retinoblastoma susceptibility gene product Rb (132). Two mechanisms underlie the E7-Rb interaction:

1. Rb interacts with active growth-suppressing members of the E2F transcription factor family. Binding of Rb interferes with the transactivation properties of E2F and also results in E2F functioning as a transcriptional repressor (133). The binding of E7 to Rb is functionally similar to Rb phosphorylation in that both result in the liberation of transcriptionally active E2F.
2. Ubiquitination and proteasome-mediated degradation of Rb result from Rb interaction with the high-risk HPV E7 proteins (HPV 16 E7/HPV 18 E7). This property of E7 correlates with its oncogenic potential. The precise mechanism whereby E7 controls Rb degradation is unclear. Perhaps E7 recruits cellular E3 ligase(s) to target Rb. Alternatively, E7 may function as an adapter between Rb and the proteasome.

E6 is sufficient for induction of immortalization of human epithelial cells. This correlates with the ability of E6 to induce degradation of p53. Both high-risk and low-risk HPV E6 proteins have been reported to bind to p53, although low-risk HPV E6 interacts with a lower affinity with p53 than does high-risk HPV E6. Only the high-risk HPV E6 induces ubiquitin-proteasome-mediated p53 degradation (134, 135). The precise mechanism is well understood (136, 137); E6 interacts with the E3 ubiquitin ligase, termed E6-AP (E6-associated protein) (138). The dimeric E6/E6-AP complex then binds p53, resulting in E6-AP-mediated ubiquitination of p53. Ubiquitinated p53 is then recognized and degraded by the proteasome. E6-AP is a member of the HECT family of the E3 ubiquitin ligases. Interestingly, mutations in E6-AP that result in inactivation of its ligase activity have been associated with a hereditary disorder termed Angelman syndrome, a neurodevelopmental disorder associated with mental retardation. Thus, HPV E6 and HPV E7 represent attractive targets for the treatment of HPV-associated malignancy. Recently, a quadrivalent HPV vaccine containing types 6, 11, 16, and 18 was developed (e.g., see 139) and recommended by the Centers for Disease Control for use in females ages 11–12 (140). This new vaccine offers great potential for widespread protection from high-risk HPV-associated cervical carcinoma.

Disordered regulation of p53 is also involved in a wide variety of malignancies. Upon activation by a variety of cellular stresses, p53 becomes stable and initiates several programs that ultimately arrest cellular proliferation. Following entry into cell cycle arrest, p53 induces repair mechanisms. If the damage is repaired, p53 becomes unstable again; if it cannot be repaired, p53 will induce apoptosis. Normally, p53 is tightly regulated, but many of the mechanisms underlying its dysregulation are currently unknown. One of the most well-defined mechanisms is its alteration of protein stability. Ciechanover et al. (141) first demonstrated p53 degradation via the ubiquitin-proteasome system, and Scheffner et al. (136) then defined the role of the E3 ligase E6-AP in

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the presence of HPV E6. Soon after, Mdm2 was identified as a p53 E3 ligase and considered the regulator of normal physiological p53 degradation. In addition, p53 was shown to drive Mdm2 expression in a negative feedback loop (142). The critical role of Mdm2 in degrading p53 is best illustrated by murine studies where inactivation of p53 is able to completely rescue embryonic lethality of loss of Mdm2 function (143). Mdm2's effects on p53 are dose-dependent, with low Mdm2 supporting p53 monoubiquitination and nuclear export, whereas high Mdm2 promotes polyubiquitination and degradation (144). In addition to Mdm2, several other E3 ligases for p53 have recently emerged. These include the E3 RING Pirh2, COP1, and ARFBP1 (145). Finally, substrate-specific p53 deubiquitinating enzymes, such as HAUSP, provide a counter regulatory mechanism to the rapid ubiquitin-dependent degradation of p53 (146).

Despite the initial discovery of the ubiquitin-proteasome system less than 30 years ago (147), a mature therapeutic has already been brought to market. Bortezomib, a tripeptide boronic acid (PS341) and highly selective proteasomal inhibitor, was the product of rational drug design directed toward selective inhibition of proteolysis of a class of proteosomal substrates (148). Given the pleiotropic role of the proteasomes within the cell, it is somewhat surprising that a general proteosomal inhibitor would demonstrate selectivity toward cancer cells both in vitro and in vivo. It is currently thought that in myeloma cells actively involved in secretion of Ig molecules, the quality control ER-associated degradation (ERAD) that removes misfolded proteins from the ER via the ubiquitin system is already saturated. Its partial inhibition by the drug leads to accumulation of misfolded proteins in the ER, induction of the unfolded protein response, and cell death. The initial clinical indication for bortezomib was in relapsed, refractory multiple myeloma (149). Bortezomib is now FDA approved for multiple myeloma patients as a second-line therapy (150). Several classes of proteasome inhibitors have been developed, including peptide aldehydes, boronic acid peptides, lactacystin and derivatives, epoxy ketones, and 2-aminobenzylstatines (151). Although inhibition of the proteasome has had demonstrable effects in animal models of inflammatory disease, arthritis, asthma, and ischemia-reperfusion injury, the efficacy in human trials has yet to be defined.

In addition to the proteasome, numerous points in the overall ubiquitin-proteasome system are attractive targets for therapeutic intervention. These have been comprehensively reviewed by Nalepa et al. (32). Only a small fraction of the genes involved in the ubiquitin pathway are currently known. Furthermore, no comprehensive evaluation has been made of the genetic alterations of these components in human disease. Thus, among the strategies ultimately attractive in regard to therapeutics, defining the genetic and genomic targets for new drugs will be important. For example, selective inhibition/inactivation of a deubiquitinizing enzyme may allow more rapid degradation of a given substrate or group of substrates. Similarly, specific targeting of an E3 ligase component will diminish ubiquitination of its substrate(s). The E3 ligases will likely provide exceptional specificity in therapeutic regulation of the ubiquitin pathway because, as described above, the pathway is hierarchical, with the greatest diversity at the E3 ligase level. Herein, there appear to be more than 300 RING-finger E3 genes in humans. One of these, MDM2, is an oncogenic RING-finger E3 for p53 (see above). Chemical library screens for MDM2 inhibitors identified nutlins (*cis*-imidazoline derivatives) that occupy the p53 binding site and thus extend p53 action, including p53-dependent cell cycle arrest. Additional targeted approaches include inhibitors of ubiquitin activation/transfer (E1/E2), specific ubiquitin-protein ligases (E3 complexes), polyubiquitin chain elongation, and the proteasome. Regulated protein targeting and turnover via the ubiquitin-proteasome system underlies a host of critical physiological and pathophysiological states in humans. The ability to modulate the individual steps in this complex pathway offers potential therapeutic strategies for the future.

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SUMMARY POINTS

1. Cellular proteins are in a dynamic state maintained by synthesis and degradation.
2. The ubiquitin-mediated proteolytic pathway accounts for the bulk of cellular protein degradation.
3. Ubiquitin-mediated protein degradation is highly regulated.
4. A wide variety of human disease states, including hypoxia, inflammatory diseases, neurodegenerative diseases, muscle wasting disorders, and cancer, involve the ubiquitin-proteasome pathway.

FUTURE ISSUES

1. The refined molecular details of the ubiquitin pathway will allow researchers to understand how a particular substrate protein is recognized by its E3 ubiquitin ligase(s).
2. The nature of the largely undefined E3 ubiquitin ligases and their substrate specificity will allow development of targeted, specific therapeutics.
3. Elucidation of the roles of this pathway in disease states will help direct therapeutic strategies.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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