

This Review is in a thematic series on **Novel Post-translational Modifications of Proteins and Their Cardiovascular Significance**, which includes the following articles:

The Emerging Characterization of Lysine Residue Deacetylation on The Modulation of Mitochondrial Function and Cardiovascular Biology [2009;105:830–841]

Protein Acetylation in the Cardiorenal Axis: The Promise of Histone Deacetylase Inhibitors [2010;106:272–284]

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Sent to Destroy: The Ubiquitin Proteasome System Regulates Cell Signaling and Protein Quality Control in Cardiovascular Development and Disease

S-Nitrosylation and Cardiovascular Signaling

S-Nitrosylation and Cardiac Ischemia

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Sent to Destroy

The Ubiquitin Proteasome System Regulates Cell Signaling and Protein Quality Control in Cardiovascular Development and Disease

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Abstract: The ubiquitin proteasome system (UPS) plays a crucial role in biological processes integral to the development of the cardiovascular system and cardiovascular diseases. The UPS prototypically recognizes specific protein substrates and places polyubiquitin chains on them for subsequent destruction by the proteasome. This system is in place to degrade not only misfolded and damaged proteins, but is essential also in regulating a host of cell signaling pathways involved in proliferation, adaptation to stress, regulation of cell size, and cell death. During the development of the cardiovascular system, the UPS regulates cell signaling by modifying transcription factors, receptors, and structural proteins. Later, in the event of cardiovascular diseases as diverse as atherosclerosis, cardiac hypertrophy, and ischemia/reperfusion injury, ubiquitin ligases and the proteasome are implicated in protecting and exacerbating clinical outcomes. However, when misfolded and damaged proteins are ubiquitinated by the UPS, their destruction by the proteasome is not always possible because of their aggregated conformations. Recent studies have discovered how these ubiquitinated misfolded proteins can be destroyed by alternative “specific” mechanisms. The cytosolic receptors p62, NBR, and histone deacetylase 6 recognize aggregated ubiquitinated proteins and target them for autophagy in the process of “selective autophagy.” Even the ubiquitination of multiple proteins within whole organelles that drive the more general macro-autophagy may be due, in part, to similar ubiquitin-driven mechanisms. In summary, the crosstalk between the UPS and autophagy highlight the pivotal and diverse roles the UPS plays in maintaining protein quality control and regulating cardiovascular development and disease. (*Circ Res.* 2010;106:463-478.)

Key Words: cardiovascular ■ development ■ ubiquitin ■ proteasome ■ signaling ■ autophagy

A growing number of studies implicate posttranslational modifications by the ubiquitin proteasome system (UPS) in regulating the complex cell signaling processes fundamental to cardiovascular development and disease.

More recently though, our understanding of the role of the UPS in protein quality control has expanded with new studies delineating its role in the endoplasmic reticulum (ER) and its cross talk with the process of autophagy. In this review, we

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Non-standard Abbreviations and Acronyms	
agLDL	aggregated low-density lipoprotein
ASA	aspirin
ASB4	ankyrin repeat and SOCS box-containing 4
ATF	activating transcription factor
CHIP	C terminus of Hsp70-interacting protein
CHOP	C/EBP homologous protein
Cul7	cullin 7 ubiquitin ligase
ER	endoplasmic reticulum
FIH	factor inhibiting hypoxia inducible factor
GRP	glucose-regulated protein
HDAC	histone deacetylase
HIF	hypoxia inducible factor
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A synthase
IRE	inositol-required enzyme
LDL	low-density lipoprotein
MDM2	murine double minute 2
MIB	mind bomb
MuRF	muscle ring finger
NF-κB	nuclear factor κ light chain enhancer of activated B cells
oxLDL	oxidized low-density lipoprotein
PERK	protein kinase R-like endoplasmic reticulum kinase
PHD	prolyl hydroxylase
SCF	Skp1, Cul1, F-box protein
TNF-α	tumor necrosis factor-alpha
UPR	unfolded protein response
UBR	ubiquitin protein ligase E3 component N-recogin
UPS	ubiquitin proteasome system
VEGF	vascular endothelial growth factor
pVHL	von Hippel-Lindau tumor suppressor

present recent evidence that suggests that the UPS plays an essential role not only in cardiovascular development but also in the dynamic pathophysiology of cardiovascular diseases (Table).

Overview of the UPS

The destruction of proteins at the cellular level is a dynamic process regulated primarily by the UPS. The UPS is a cascade of carefully regulated enzymes which consist of E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) enzymes (see Figure 1A), which target proteins for destruction by the proteasome. Each of these enzymes (E1, E2, and E3) plays a unique role in the posttranslational modification of specific proteins. The E1 uses ATP to generate a high energy thioester bond with ubiquitin between catalytic cysteine residues within E1 and the c-terminal glycine residue of ubiquitin. This “activated” ubiquitin is then available to be transferred to one of the ubiquitin conjugating enzymes (E2). There are dozens of these E2 enzymes to which the charged ubiquitin is added. These E2 enzymes then interact with one of the hundreds

of ubiquitin ligases (E3) to transfer the activated ubiquitin to the ϵ -amino group of a lysine residue in the target protein. The ubiquitin on the target protein then serves as an “acceptor” on which additional ubiquitins can be added. Several cycles of this ubiquitin ligase activity results in a polyubiquitin chain being formed on the target protein.

Ubiquitin is a 76-amino acid moiety with multiple lysines capable of making isopeptide linkages to form polyubiquitin chains (Figure 1B). This allows a diversity of polyubiquitin chain configurations that can drive different fates for the proteins to which they are attached. The most commonly identified polyubiquitin linkage occurs on the lysine at amino acid 48 (Lys48). Polyubiquitin chains that link through its Lys48 are called canonical ubiquitin chains and target the ubiquitinated substrate for destruction. However, there are a total of seven lysine moieties in ubiquitin which can be used for chain formation (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63).¹ Conformationally, polyubiquitin chains formed through Lys63 have been identified to be more linear than chain made through Lys48 as illustrated in Figure 1B. Lys63 polyubiquitin chains can modify the activity of the target proteins² and have been implicated in DNA repair mechanisms³ and in the regulation of physiological cardiac hypertrophy.² The significance of the addition of polyubiquitin chains formed through the noncanonical lysines (Lys6, Lys11, Lys 27, Lys29, Lys33) has not been completely elucidated. The addition of a single ubiquitin on a protein, called monoubiquitination, regulates DNA repair, nuclear export, and histone regulation. In the heart, several signaling processes are regulated by monoubiquitination, including receptors involved in signaling pathways (EGF),⁴ cell-to-cell electric coupling (connexin 43),^{5,6} apoptosis (caspase 3 and caspase 7),⁷ and calcium regulation (via calmodulin).⁸ The posttranslational modification of a protein by poly- or monoubiquitination may determine its fate and regulate its activity. For example, polyubiquitin chains may target misfolded proteins for degradation, whereas monoubiquitination may tag these same proteins for delivery to other cellular compartments effectively inhibiting their activity.⁹ Deubiquitinating enzymes counteract the ubiquitination process (Figure 1A). The nearly 100 described deubiquitinating enzymes cleave ubiquitin and help recycle ubiquitin removed at the 26S proteasome as ubiquitinated proteins are degraded.¹⁰ Not only can deubiquitinating enzymes reverse the modification of ubiquitinated proteins, they play a role in the remodeling of polyubiquitin chains.¹⁰

The UPS Regulates Cell Signaling in Vascular Development

Development of the vascular system is one of the earliest and most pivotal events that occur during embryogenesis. The development of blood vessels de novo occurs when mesodermally derived angioblasts differentiate to form primitive blood vessels (vasculogenesis). Sprouting and bridging of this primary plexus occurs through angiogenesis, where endothelial cell outlines are covered by smooth muscle cells in the large vessels. Arteriogenesis, the process of remodeling existing capillaries in response to increased flow demand, is

Table. Proteins Involved in the UPS Regulation of Cardiovascular Development and Pathophysiology of Cardiovascular Diseases

Proteins	Function	Role
Vascular development		
Numb	Notch antagonist	Ubiquitinated by LNX, preventing internal sequestration of Notch
Itch	Ubiquitin ligase	Polyubiquitinates Notch in absence of a ligand, which promotes endocytosis and inhibition of Notch
FBW7/Sel-10	Ubiquitin ligase	Targets Notch for proteasomal degradation during vascular development
MIB (mind bomb) family	Ubiquitin ligase	Regulates Notch ligand signaling (MIB1—developing embryo; MIB2—adult tissues)
Nedd-4	Ubiquitin ligase	Targets VEGF-receptor 2 for proteasomal degradation
VHL (von Hippel-Lindau)	Ubiquitin ligase	Regulates hypoxia inducible factor 1 (HIF1) to adapt to low oxygen concentrations
CHIP (C terminus of Hsc70-interacting protein)	Ubiquitin ligase	Targets myocardin, FOXO1; mediates smooth muscle cell differentiation and proliferation
HIF1 α	Ubiquitin ligase/transcription factor	During hypoxia transcribes pro-angiogenic factors e.g. VEGF, TGF- β 3
FIH	Hydroxylation factor	Binds HIF1 to make it transcriptionally inactive
Siah2	Ubiquitin ligase	Promotes HIF1 activity at specific oxygen concentrations
VDU2	VHL deubiquitinase	Stabilizes HIF1 by deubiquitination
ASB4 (ankyrin repeat SOCS box protein 4)	Ubiquitin ligase	Interacts directly with FIH; high levels expressed with drastic increases in oxygen tension
FBW7, Cul 7	Substrate recognition protein/SCF (Skp1, Cul1, F-box protein) type ubiquitin ligase	Required for cardiovascular development; implicated in cancers (targets destruction of oncogenes e.g. myc, c-Jun, Notch, cyclin E)
Cullin	SCF type ubiquitin ligase	Deletion results in hemorrhagic vasculature and abnormal placental endothelial differentiation
Atherosclerosis		
Proteasome inhibitors		Reduce endotoxin-induced gene expression; prevent LPS-induced inflammatory responses; upregulate nitric oxide synthase in endothelial cells
TNF α , NF- κ B	Inflammatory mediator/transcription factor	Correlated with protein ubiquitination and 20S proteasome activity
CYLD	Deubiquitinating enzyme	Inhibits TNF α -induced NF- κ B activation and expression of Cyclin D1 through deubiquitination of TNFR-associated factor 2 (TRAF2) and Bcl-3
PAF (platelet activating factor)	Inflammatory mediator	Downregulated by lysosomal- and ubiquitin-dependent proteasomal-mediated receptor degradation
CRP	Acute phase protein	Transgenic expression of human CRP reduced atherosclerotic lesion size and was associated with increased plaque expression of 26S proteasome subunits
LIG (lipoprotein-inducible gene)	Human homologue of bovine ubiquitin-conjugating (E2) enzyme	Increased during aggregated LDL challenge of macrophages
HMG-CoA	Cholesterol synthesis regulatory enzyme (hepatocyte)	Turnover mediated by ubiquitination and activity of 26S proteasome
Idol	Ubiquitin ligase	Regulates LDL receptor
COP9 signalosome		Mediates ABCA1 (ATP-binding cassette protein A1) degradation

(Continued)

Table. Continued

Proteins	Function	Role
Cardiovascular Biology		
clAP, XIAP	Degrade caspases	Regulation of apoptosis
MDM2, COP1, Pirh2, ARF-BP1, CHIP	Degrade p53	Regulation of apoptosis
MuRF1, CHIP, MAFBx/atrogin-1, MDM2	Ubiquitin ligases	Pathophysiology of various cardiac diseases
ER-associated degradation, ER stress, UPR		
PERK, ATF6, IRE-1	ER transmembrane proteins	Mechanisms by which ER senses stress (Unfolded Protein Response)
GRP78	ER chaperone	In normal times, binds to internal surfaces of PERK, ATF6, IRE-1 and blocks downstream signaling
GRP94	SR chaperone	Overexpression protects against Ca ²⁺ overload, ischemia-induced cell death
Puma (p53-upregulated modulator of apoptosis)	Modulates apoptosis	Upregulated during induced ER stress and UPR
Cardiac development		
N-recognins: UBR1, UBR2	Ubiquitin ligases	Mediate the N-end rule pathway by recognizing N-degrons
Cardiac Disease		
MuRF1, MuRF2, MuRF3, MAFBx/Atrogin-1, CHIP, MDM2	Ubiquitin ligases	Mechanistically characterized in hypertrophy and ischemia reperfusion injury
Selective autophagy		
HSP-E3 complex	Protein quality control	Promotes folding of misfolded proteins, enhances ubiquitination, and targets misfolded proteins for proteasome degradation
Atg12-Atg5; LC3(Atg8)-PE	Autophagy pathways	Conjugate to Atg proteins forming complexes essential for recruitment of LC3 and formation of autophagosome membranes
p62	Intracellular receptor, adaptor protein	Recognizes ubiquitin chains on targeted proteins and delivers them to autophagosomes; also regulates clearance of proteins by UPS
NBR1	Intracellular receptor, adaptor protein	Mediates cross-linking of ubiquitinated proteins; interacts w/p62 to clear misfolded proteins
HDAC6	Intracellular receptor, adaptor protein	Recognizes ubiquitinated/misfolded proteins shuttling them into aggresomes; delivers for autophagy via microtubule organizing centers (MTOC)
BAG3	Hsc/Hsp10 co-chaperone	Mediates autophagic degradation of ubiquitinated proteins in aging cells; co-localizes with p62-positive aggregated proteins
BAG1		Mediates proteasome-dependent degradation of ubiquitinated proteins w/CHIP

also fundamentally involved in vascular development. These processes all regulate precursor cells in the developing embryo as well as in the adult through common signaling pathways such as Notch, vascular endothelial growth factor (VEGF), and hypoxia inducible factor (HIF)1 α among others.^{11,12} In turn, each of these signaling pathways can be and are regulated by the UPS.

UPS Regulation of Notch Signaling

The UPS regulates Notch signaling by its interaction with and regulation of the Notch antagonist Numb. The ubiquitin ligase LNX (ligand of numb-protein X) can ubiquitinate

Numb, preventing internal sequestration of Notch, resulting in enhanced downstream Notch signaling.¹³ The ubiquitin ligase Itch can polyubiquitinate Notch in the absence of a ligand, promoting endocytosis and inhibition of Notch.¹⁴ Furthermore, during vascular development the ubiquitin ligase FBW7/Se1-10 can target Notch to the proteasome for degradation.¹⁵ Adding to the complexity of UPS-mediated regulation of Notch is the fact that Notch ligands are also targets of proteasomal degradation. Two studies have demonstrated that the mind bomb (MIB) family of ubiquitin ligases serves a regulatory role in Notch ligand signaling, with individual family members temporally restricted in

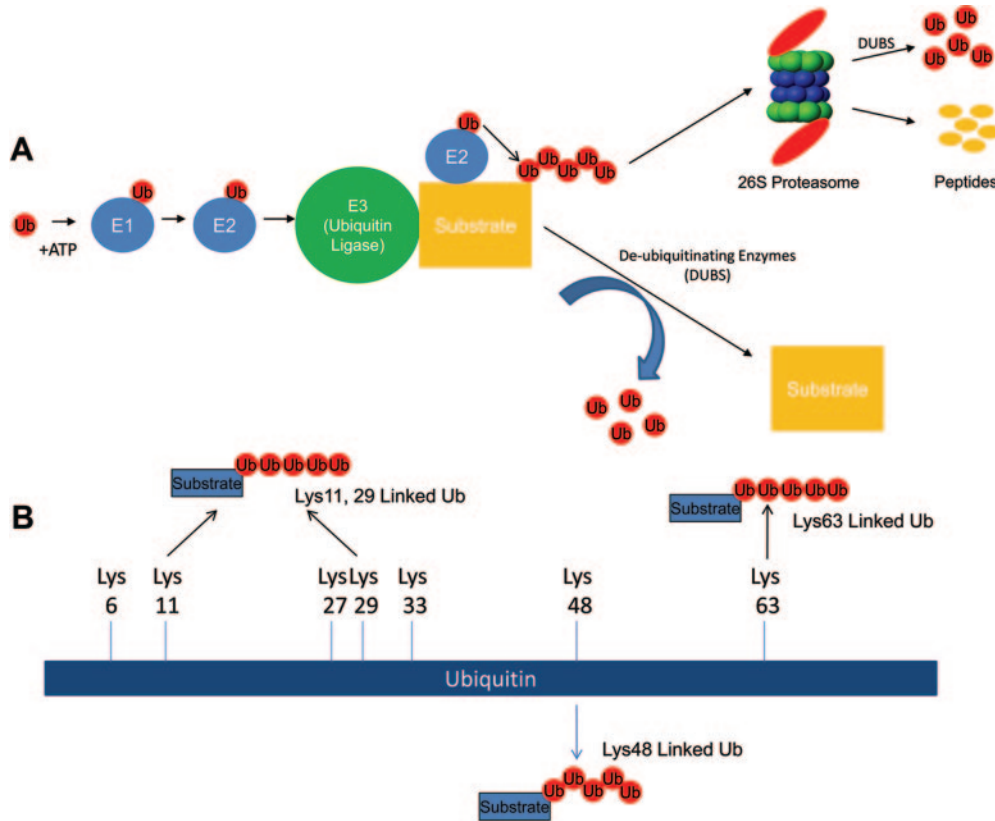


Figure 1. The ubiquitin proteasome system at a glance. The ubiquitin proteasome is a system of enzymes that places ubiquitin (chains) on specific protein substrates to target them for degradation, change their localization, and/or enhance their activity. A, Free monoubiquitin is activated by the E1 enzyme in an ATP-dependent manner and transferred to the E2 enzyme. The specificity of the system is in the E3 (ubiquitin ligase) that mediates the transfer of one or more ubiquitin moieties sequentially to form ubiquitin chains on the substrate. The canonical lysine chains linked by their lysine 48 (Lys48) are recognized by the 26S proteasome, which degrades the protein into constituent peptides and free ubiquitin. B, The role of noncanonical polyubiquitination (ie, Lys63-linked ubiquitin chains) is increasingly being described in the cardiovascular system as a way to regulate protein (ie, transcription factors) activity. A total of 7 lysine moieties exist in ubiquitin, which can be used for chain formation (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63). A number of studies discussed in this review reveal a role for more linear Lys63 chains, in addition to canonical Lys48 linkages in the cardiovascular system. However, the significance of Lys6, Lys11, Lys27, Lys29, Lys33, and branching/complex ubiquitin chains has not been elucidated in general.

expression to either revascularizing adult tissue (MIB2) or the developing embryo (MIB1).^{16,17}

UPS Regulation of VEGF Signaling

Perhaps the most widely studied component of vascular development is VEGF signaling. VEGF signaling is crucial for angiogenesis, vasculogenesis, cell migration, proliferation, and cell survival.¹⁸ Mice lacking VEGF die at approximately embryonic day 8.5 and have significant impairments in angiogenesis and blood-island formation.¹⁹ On the opposite end of the spectrum, even modest increases in VEGF disrupt vascular development.²⁰ Improper regulation of VEGF has also been implicated in the pathophysiology of pulmonary inflammatory disease, cancer proliferation, diabetic retinopathy, and rheumatoid arthritis. The VEGF receptor 2 can be ubiquitinated by Nedd-4, targeting it to the proteasome for degradation.²¹ However, this Nedd-4 mediated regulation can itself be regulated by its association with Grb10.²¹ VEGF is also regulated by oxygen sensing mechanisms; the ubiquitin ligase VHL regulates HIF1 to adapt to low oxygen concentrations.

UPS Regulation of Smooth Muscle Cell Development

Another major component of vascular development involves the development of vascular smooth muscle cells (SMCs). The precise coordination of proliferation and differentiation of SMCs is required for proper vasculature. Recent studies indicate that the ubiquitin ligase CHIP (C terminus of Hsc70-interacting protein) mediates both SMC differentiation and proliferation through ubiquitination and proteasomal destruction of specific substrates. Targeting by CHIP of myocardin, a key cotranscription factor of serum response factor, decreases SMC differentiation.²² However, when CHIP targets FOXO1, a repressor of SMC differentiation, the subsequent FOXO1 repression is ameliorated, thus mitigating apoptosis and enhancing SMC growth.²³ Other ubiquitin ligases (ie, Skp2, MDM2 [murine double minute 2]) have been described in SMC biology, but their role in differentiation has yet to be explored.^{24,25}

HIF1 α As a Prototypic Transcription Factor Regulated by the UPS in Vascular Development

The best characterized ubiquitin ligase that regulates vascular development is HIF1. The HIF1 signaling cascade mediates

the necessary adaptations the vasculature needs to make in the presence of low oxygen concentrations, including the formation of vessels in both embryos and adults. HIF1 is a dimeric transcription factor composed of an α and β subunit. Under normoxic conditions, HIF1 α subunits are rapidly degraded by the UPS. However, in response to decreased oxygen levels, HIF1 α becomes stabilized and transcribes an array of proangiogenic factors, such as VEGF, transforming growth factor- β_3 , and various components of glucose transport and glycolysis, which are generally thought to overcome vascular insufficiency.^{26–28} Although regulation of HIF1 can occur at the mRNA level,²⁹ it is widely believed its primary regulation is through posttranslational modification and degradation of HIF1 α via the UPS.

The von Hippel-Lindau tumor suppressor protein (pVHL) is an essential part of the ubiquitin ligase complex that negatively regulates HIF1 α . During periods of normoxia, prolyl hydroxylase (PHD) catalyzes the hydroxylation of the HIF1 α subunits on conserved proline residues, converting them into hydroxyproline. This in turn is recognized by the pVHL ubiquitin ligase complex resulting in HIF1 α ubiquitination and subsequent degradation by the proteasome. A second form of HIF1 inhibition occurs through its interaction with the factor inhibiting HIF (FIH). FIH binds HIF1 and hydroxylates an asparagine residue on the C-terminal transactivation domain of HIF1. This hydroxylation prevents the coactivator p300/CBP from associating with the C-terminal transactivation domain of HIF1, rendering it transcriptionally inactive. Although apparently redundant, it has recently become clear that FIH and PHD-pVHL modes of HIF1 inhibition operate in segregation as well, depending on the precise oxygen gradient, as evidenced by mathematical and biological predictions.³⁰ Although this canonical view of HIF1 regulation by pVHL and FIH is generally believed to be the predominant modulator in oxygen-mediated vascular development, other elements exist adding further layers of complexity. For example, by negatively regulating the activity of PHD enzymes, the ubiquitin ligase Siah2 promotes HIF1 activity at specific O₂ concentrations.³¹ Additionally, the VHL deubiquitinase VDU2 is able to stabilize HIF1 α by deubiquitinating it, resulting in the enhancement of HIF1 activity.³²

Another crucial factor involved in vascular differentiation and development regulated by FIH is the ankyrin repeat SOCS box protein 4 (ASB4), originally identified as a ubiquitin ligase that is differentially expressed in vasculature lineages in embryoid bodies.³³ ASB4 interacts directly with FIH and is itself a substrate of FIH-mediated hydroxylation, the results of which may promote binding to and degradation of substrates of ASB4.³³ High levels of ASB4 are expressed in the embryonic vasculature at times when drastic increases in oxygen tension occur (embryonic day 8.5 to 9.5).³³ Additionally, *in situ* mRNA distribution analysis reveals that ASB4 is spatially compartmentalized to the developing capillary plexi, intersomitic vessels, and placenta.³³ As the vasculature matures and oxygen levels are stabilized, ASB4 expression is downregulated, further implicating its role in differentiation. ASB4 makes up a ubiquitin ligase complex with elongin B/elongin C/cullin/Roc and serves to give the

complex its specificity.³³ Overexpression of ASB4 promotes differentiation of vascular precursors into the vascular lineage in an oxygen-dependent manner, strengthening its proposed role in vascular development.³³

Other UPS Components Linked to Signaling in Vascular Development: FBW7 and Cul7

The substrate recognition protein FBW7 (F-box and WD repeat domain-containing 7) is part of a conserved SCF (Skp1, Cul1, and F-box protein)-type ubiquitin ligase complex. FBW7 is required for cardiovascular development, because FBW7-null mice die at embryonic day 11 from impaired cardiac and vascular development.^{15,34} Conditional knockouts in hematopoietic stem cells results in a decrease in all lineages of blood cells (pancytopenia)³⁵ and deletion of FBW7 in mouse T-cells, disrupting their cell-cycle exit.³⁶ Conditional FBW7 knockouts can also develop leukemia. This is not surprising because FBW7 is implicated in many human cancers, targeting the destruction of oncogenes such as *myc*, *c-Jun*, *Notch*, and *cyclin E*.³⁵ Cullin proteins is another component of the SCF ubiquitin ligase complex that has been linked to the developing vasculature. Arai et al identified that the deletion of the Cul1 homolog p185 (Cul7 [cullin 7 ubiquitin ligase]) results in neonate lethality from respiratory distress.³⁷ They further show that embryos exhibit hemorrhagic vasculature, and abnormal placental endothelial differentiation and vascular structure. Interestingly, in the same report Cul7 was also shown to form a SCF-like complex that includes glomulin (aka FAP48 and FAP68), which has been suggested as the loss-of-function component in familial glomuvenous malformation. This hereditary syndrome is characterized by abnormalities in vascular morphology and morphogenesis.³⁸

The Role of the UPS in the Pathophysiology of Atherosclerosis

Recent studies have identified the contribution the UPS makes to the development of atherosclerosis by regulating vascular inflammation, oxidative stress, apoptosis, and cholesterol metabolism. These studies, outlined below, provide evidence that implicates the UPS in many diverse mechanisms that contribute to the pathogenesis of atherosclerosis.

The UPS Regulates Vascular Inflammation and Oxidative Stress Responses

Present dogma states that atherosclerosis is, in large part, an inflammatory disease of the vessel wall. Several reports provide insight into how the UPS may affect specific components of the inflammatory response and may therefore modulate the atherosclerotic process. The UPS plays a role in modifying the function of inflammatory and vascular cells. Regulatory T cell (T_{reg}) function plays a role in attenuating the inflammatory/immune component of atherogenesis. Meier et al have shown that treatment of T_{reg} cells with oxidized low-density lipoprotein (LDL) or uremic serum causes a reduction in proteasomal activity that leads to cell cycle arrest and apoptosis.³⁹ They postulate that the resulting immune dysfunction exacerbates inflammation and athero-

genesis in patients, especially those with end stage renal disease.³⁹ The macrophage, central to the development of inflammation and atherosclerosis, is dependent, in part, on its proteasome activity to function. Proteasome inhibitors reduce endotoxin-induced gene expression including the Toll-like receptor 2 and can prevent lipopolysaccharide-induced inflammatory responses.⁴⁰ Thus, regulation of the macrophage proteasome activity can modulate the function of these cells and may therefore alter the atherogenic process. Endothelial cell function is also intimately involved in atherogenesis, and regulation of endothelial proteasome activity can modulate the inflammatory phenotype. For example, proteasome inhibition causes the upregulation of endothelial nitric oxide synthase in endothelial cells and thereby enhances endothelial-dependent vasorelaxation of rat aortic rings.⁴¹ Thus, proteasome function may be necessary to induce the endothelial cell dysfunction that contributes to atherosclerosis, other vascular diseases, and systemic diseases characterized, in part, by vascular dysfunction.

The UPS can also modify the function of various inflammatory mediators. Tumor necrosis factor (TNF) α stimulates inflammation and immune responses in part through activation of nuclear factor (NF)- κ B, a nuclear transcription factor that plays an important and central role in the generation of inflammation, apoptosis and cell proliferation. In carotid artery plaques from patients with various clinical characteristics, protein ubiquitination and 20S proteasome activity is correlated with the presence of TNF α and NF- κ B.^{42–44} Plaques from patients with morning surges in blood pressure have increased protein ubiquitination and 20S proteasome activity, which is associated with increases in NF- κ B, TNF α , inflammatory cell number, markers of oxidative stress, and matrix metalloproteinase 9 but decreases in collagen content and I κ B levels compared to controls.⁴³ Similar findings have been reported in plaques from postmenopausal women not receiving hormone replacement therapy compared to those who are⁴⁴ and in plaques from diabetics not treated with rosiglitazone compared to those who were treated.⁴² These findings demonstrate that protein ubiquitination and 20S proteasome activity is associated with inflammation, oxidative stress, and histological changes leading to an unstable plaque phenotype. TNF α also stimulates an increase in the expression of the deubiquitinating enzyme cylindromatosis (CYLD) in endothelial and smooth muscle cells. CYLD inhibits TNF α -induced NF- κ B activation and expression of Cyclin D1 through deubiquitination of TRAF2 (TNF receptor-associated factor 2) and Bcl-3, respectively. Overexpression of CYLD inhibits cell viability and neointima formation in a rat model of carotid artery injury.⁴⁵ A recent study assessing the role of the UPS in atherosclerosis in rabbits investigated how inhibiting the 20S proteasome by aspirin (ASA) affected atherosclerosis progression. Rabbits were fed a high fat diet and some were additionally treated with ASA. In ASA-treated rabbits, atherosclerotic lesions were less apparent and more ubiquitinated proteins were present. Signaling through NF- κ B was inhibited by ASA, as determined by a number of measures, suggesting that the therapeutic effect of ASA

may be due, in part, to the inhibition of the proteasome and subsequent degradation of I κ B.⁴⁶

Platelet-activating factor is a potent mediator of inflammation thought to be important in atherogenesis.⁴⁷ However, its receptor-stimulated activity is characterized by rapid desensitization attributable to receptor down-regulation. This down-regulation is attributable to lysosomal- and ubiquitin-dependent proteasomal-mediated receptor degradation,⁴⁸ suggesting that inhibition of the UPS may exacerbate the effects of platelet-activating factor on vascular cells and be proatherogenic. Finally, C-reactive protein, an acute phase protein increased in inflammation, has been postulated to contribute to atherogenesis.⁴⁹ A recent study in a strain of atherosclerosis-prone mice that are LDL receptor-deficient and express ApoB100 demonstrated that transgenic expression of human C-reactive protein reduces lesion size and is associated with increased aortic plaque expression of several subunits of the 26S proteasome. These include genes for the 20S subunit (PSMA7, PSMB7, PSMB9), the 19S cap unit (PSMC6), and the 11S cap unit (PSME2).⁵⁰ These studies provide evidence that the UPS may modulate the activity and/or function of many of the inflammatory mediators and cell types that participate in mechanisms of atherogenesis. Given the large number of possible targets and variation of effects the UPS system has on substrates, predicting the overall role the UPS has on atherosclerosis will be difficult. This may explain why therapeutic proteasome inhibition has made atherosclerosis both better and worse, depending on the model.^{42–44,51–54}

UPS Regulation of Vascular Cell Apoptosis

Certain aspects of lipoprotein metabolism are important in atherosclerosis and may be modulated by the UPS. It is well accepted that modified forms of LDL are risk factors for atherosclerosis. Studies of the effects of aggregated LDL (agLDL) on macrophages have shown that challenge with agLDL triggers polyubiquitination of intracellular proteins and ubiquitin-dependent degradation of the apoptosis inducer p53. There is a concomitant increase in expression of LDL-inducible gene (LIG), a human homolog of the bovine ubiquitin-conjugating (E2) enzyme E2-25K. Inhibiting the proteasome blocks this antiapoptotic effect of agLDL, and increases the half-life of p53. Thus, agLDL may protect macrophages from apoptosis in a LIG- and proteasome-dependent manner.⁵⁵ Other studies have found a role for the UPS in oxidized LDL (oxLDL)-induced apoptosis of vascular cells. OxLDL-induced ubiquitination of cellular proteins induces early activation but late depression of proteolysis. Proteasome inhibition exacerbates the toxicity of oxLDL, demonstrating that the UPS may be involved in oxLDL-induced apoptosis.⁵⁶ OxLDL also downregulates insulin-like growth factor-1 receptor, leading to smooth muscle cell apoptosis.⁵⁷ Higashi et al⁵⁸ has demonstrated that this down-regulation is dependent on enhanced Nedd4-dependent receptor ubiquitination, but that degradation of the receptor is independent of the proteasome pathway. In contrast to earlier studies, these later reports suggest that ubiquitination and proteasomal activity may enhance apoptosis of certain vascular cell types. Thus, the role of the UPS in cellular

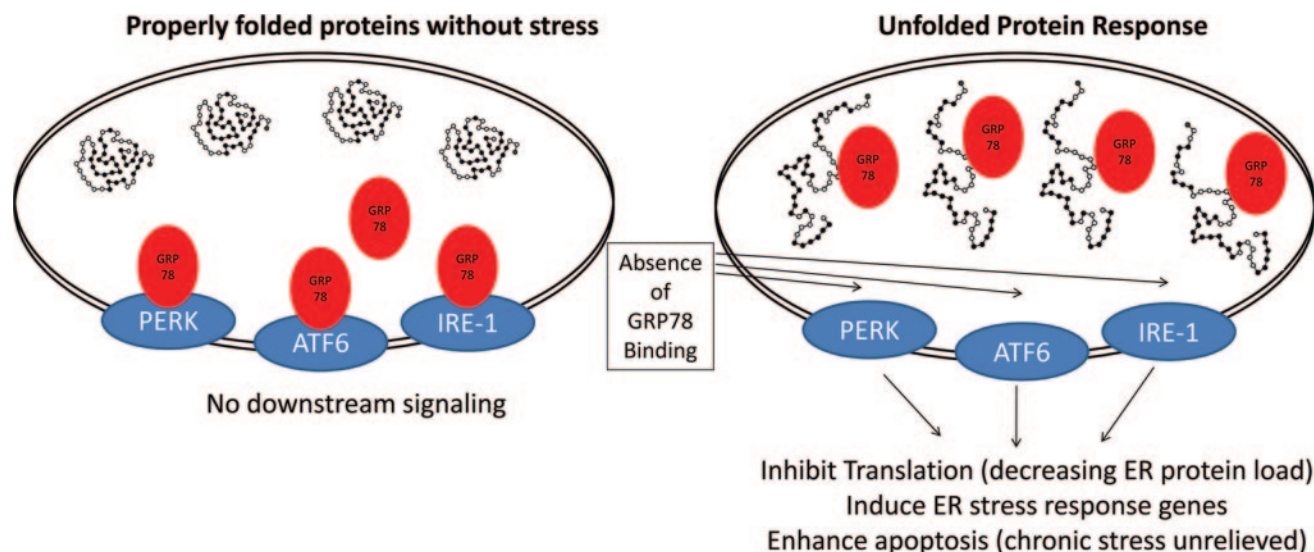


Figure 2. Endoplasmic reticulum stress and the UPR. In response to stress, proteins synthesized in the rough ER are refolded by resident molecular chaperones GRP78. On refolding, GRP78 loses its association with the luminal domains of the PERK, ATF6, and IRE-1. This leads to PERK, ATF6, and IRE-1 activation and downstream activation of ER stress response genes, which help stabilize the misfolding of proteins, including ER-targeted chaperones. If the unfolded proteins are not adequately removed after the activation of the UPR, signaling pathways for apoptosis can be activated. Adapted from Glembotski.^{79,80}

apoptosis and its contribution to atherogenesis may vary depending on the mechanism of apoptosis and target cell type.

UPS Modulation of Cholesterol Metabolism

Cholesterol metabolism and transport is central to the development of atherosclerosis, and there is evidence that the UPS may play a role in these processes. HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A synthase) is a key regulatory enzyme of cholesterol synthesis in the liver, and pharmacological inhibition of HMG-CoA has been very successful in lowering blood cholesterol concentrations. Studies show that sterol-stimulated turnover of HMG-CoA is mediated by its ubiquitination as well as activity of the 26S proteasome,⁶⁰ implicating the UPS in (dys)regulation of cholesterol production for peripheral tissues. The LDL receptor itself has recently been shown to be regulated by ubiquitin-dependent degradation by the ubiquitin ligase Idol (inducible degrader of the LDL receptor), which is transcriptionally regulated by nuclear receptors to maintain cholesterol homeostasis.⁶¹ Overexpressing Idol promotes LDL receptor degradation and elevates plasma LDL levels demonstrating its potential anti-atherogenic role.⁶¹ Reverse cholesterol transport from peripheral tissues back to the liver is mediated by high-density lipoprotein, and loading of high-density lipoprotein with cholesterol is mediated by ATP-binding cassette protein A1 (ABCA1). Work by Azuma et al⁶² suggests that ABCA1 degradation is mediated by the COP9 signalosome and is a key controller of ubiquitination. Therefore, the UPS may modulate atherogenesis, in part, through these several mechanisms involving the regulation of cholesterol metabolism.

UPS and Cardiovascular Biology

The UPS regulates a wide array of biological processes in the cardiovascular system (see recent reviews^{63–65}). For example,

the UPS plays a role in the regulation of voltage-gated channels, including the hERG1 channel linked to familial long QT syndromes. Many of the more than 200 mutations in this gene lead to misfolded proteins that are rapidly degraded by the ER-associated degradation pathway (discussed below).^{66,67} The UPS also regulates a number of signal transduction pathways and transcription factors. The UPS has a significant role in attenuating mitogen-activated protein kinase activation,⁶⁸ signaling through NFAT via calcineurin,^{69,70} and regulating NF- κ B signaling.⁷¹ The UPS also regulates apoptosis directly through the degradation of caspases (by cIAP, XIAP) and p53 (by MDM2, COP1 [constitutive photomorphogenic 1 protein], Pirh2 [p53-induced protein with a RING-H2 domain], ARF-BP1, CHIP).⁶³ Additional ubiquitin ligases specifically regulate the pathophysiology of cardiac diseases, including muscle ring finger (MuRF)1, CHIP, MAFBx (muscle atrophy F-box)/atrogen-1, and MDM2. The number and diversity of processes the UPS regulates in the cardiovascular system continues to increase, such that the role of UPS is now recognized as intimately associated with ER stress, ER-associated degradation, and autophagy in the cardiovascular system.

ER-Associated Degradation, ER Stress, and the Unfolded Protein Response in the Cardiovascular System

The unfolded protein response (UPR) is a signal transduction system activated in response to stresses that affect the ability of the ER to properly fold proteins. This system is activated in response to increased protein misfolding (Figure 2). Several factors are necessary to maintain efficient protein folding in the ER including the maintenance of the redox state, maintenance of the glycosylation machinery, as well as chaperones to optimally fold newly formed proteins in the ER. The ER senses stress by at least

3 transmembrane proteins: (1) the protein kinase R-like ER kinase (PERK); (2) activating transcription factor (ATF)6; and (3) the inositol-requiring enzyme-1 (IRE-1).^{72–78} With normal functioning, the ER chaperone glucose-regulated protein (GRP)78 bind to the internal ER surfaces of PERK, ATF6, and IRE-1 effectively blocking downstream signaling through these receptors (Figure 2). When normal protein folding is disrupted, GRP78 accumulates with the misfolded proteins in the ER in an apparent attempt to refold the proteins (Figure 2).^{72–76} Without GRP78 to bind the UPR receptors in the ER, the three ER receptors mediate downstream signaling in response to the increase in ER stress. The distal effectors of these 3 UPR receptors then mediate an arrest in protein biosynthesis (translation), increased expression in ER responsive genes (including chaperones, calcium binding proteins, and disulfide isomerases), and effectors of apoptosis (through CHOP and c-Jun NH₂-terminal kinase/caspase-12), as recently reviewed.^{79,80} Through these mechanisms, the UPR can exert both positive and negative influences on cell survival.

ER Stress Signaling Pathways in the Heart: UPR-Mediated Protection

The induction of the UPR (or components thereof) protect against ischemic challenge in cardiomyocytes. Hearts from transgenic mice with cardiac-specific ATF6 overexpression are protected against ischemia/reperfusion injury, suggesting that ATF6-mediated signaling upregulates proteins that protect against cell death.⁸¹ In cultured cardiomyocytes, increasing the expression of GRP78 during preconditioning imparts protection.^{82,83} Similarly, increasing the sarcoplasmic reticulum chaperone GRP94 in C2C12 myocytes or H9C2 cardiomyocytes protects against Ca²⁺ overload or ischemia-induced cell death determined by propidium iodide uptake.⁸⁴ GRP94 overexpression in neonatal cardiomyocytes also results in protection against simulated ischemia. The UPR in cultured cells can be induced experimentally using the antibiotic tunicamycin. Tunicamycin-evoked UPR results in an increase in GRP78 in H9C2 cardiomyocytes, and subsequent protection against simulated ischemia/reperfusion.⁸⁵

ER Stress Signaling Pathways in the Heart: UPR-Mediated Apoptosis

The UPR also plays a critical role in mediating apoptosis and cell death in response to cardiac ischemia/reperfusion injury. Activation of the UPR can induce apoptosis, whereas inhibiting it may protect against ischemia/reperfusion-induced cell death. For example, tunicamycin challenge in cultured cardiomyocytes induces the rapid translocation of δ -protein kinase C (δ PKC) and subsequent cell death, determined by assays for necrosis (LDH release) and apoptosis (caspase activation, TUNEL staining).⁸⁶ However, if δ PKC activation is inhibited, tunicamycin-induced cell death is reduced, as are other specific indicators of UPR, such as GRP78 expression and c-Jun NH₂-terminal kinase phosphorylation.⁸⁶ Similarly, blockade of δ PKC activation resulting from ischemia/reperfusion injury-induced UPR protects against induced cell

death.⁸⁶ Because the protein Puma (p53-upregulated modulator of apoptosis) is required for the induction of cardiomyocyte cell death in ischemia/reperfusion injury,⁸⁷ recent studies have investigated its role in the UPR response. Upregulation of Puma and increased apoptosis are seen in rat and mouse neonatal cardiomyocytes that have been treated with thapsigargin or tunicamycin to induce ER stress and the UPR.⁸⁸ Inhibiting Puma significantly protects these cardiomyocytes from ER-stress induced apoptosis, suggesting the critical role of Puma in mediating ER-induced cardiomyocyte death.⁸⁷

Temporal Regulation of the ER Stress Response

The ER stress response may be temporally controlled according to recent studies. When neonatal cardiomyocytes undergo apoptosis in response to simulated ischemia or serum/glucose/oxygen deprivation, activation of the UPR precedes caspase activation. The initial response of cardiomyocytes to these stresses is the activation of the UPR, as assessed by increases in GRP78, XBP1, and eIF2 α phosphorylation.⁸⁹ At later time points, the UPR activation transitions to the activation of CHOP and procaspase 12 processing.⁸⁹ These studies suggest that UPR effectors initially try to maintain protein quality by increasing the folding capacity (eg, increasing the GRP78 chaperone) and later induce apoptosis if they are unable to overcome the stress. The downstream signaling processes in the cardiomyocyte UPR are obviously complex given the number of receptors and effectors involved (Figure 2). The cardiac UPR is also activated by the induction of diabetes by streptozocin⁹⁰ and the use of proteasome inhibitors.⁹¹ With the growing prevalence of diabetes and the increased use of proteasome inhibitors, understanding the ER stress response in the heart may be increasingly relevant to cardiac health in diseases not primarily of cardiac origin.

The Role of Ubiquitin Ligase N-Recognins in Cardiac Development

Recent studies have identified ubiquitin ligases that recognize structural motifs or degradation signals (“degrons”) within target proteins present within the substrate structure. A degron is defined as the minimal part of a protein sufficient for recognition and degradation. There are 3 components of the N-degron signal in eukaryotic proteins: (1) a destabilizing N-terminal residue; (2) its internal lysine residue(s) where the polyubiquitin chain forms; and (3) the conformational flexibility of areas around these determinants.^{92–95} Recent studies have identified that a family of ubiquitin ligases, called N-recognins, mediate the N-end rule pathway.^{92,96,97} Mammalian N-recognins have been identified that recognize N-degrons: UBR1 and UBR2 (ubiquitin protein ligase E3 component n-recognin 1 and 2).^{98,99} These components of the N-end rule pathway are essential for proper cardiac development as evidenced by the wide range of cardiovascular abnormalities seen in UBR1- and UBR2-deficient mice.

The Ubiquitin Ligases UBR1 and UBR2 Are Necessary for Cardiac Development

UBR1 and UBR2 have indistinguishable patterns of binding to N-degrons⁹⁸; however, their *in vivo* roles do not clearly overlap.¹⁰⁰ Mice lacking UBR1 (UBR1^{-/-}) are viable and fertile, exhibiting only a mild hypoglycemia, disturbed fatty acid synthase activity, and exocrine pancreatic insufficiency.^{101,102} Mice lacking UBR2 (UBR2^{-/-}) exhibit gender specific defects: males are viable but infertile, whereas females die as embryos.⁹⁸ The differences in the apparent role of UBR1 and UBR2 in development may be attributable to differential expression patterns in cell types and tissues, although this has yet to be specifically tested. A better understanding of the role of UBR1 and UBR2 in development has been achieved by the creation of UBR1^{-/-}/UBR2^{-/-} double-null mice.¹⁰⁰ These mice die at midgestation with defects in cardiovascular development. Unlike single UBR1^{-/-} or UBR2^{-/-} mice, double-null mice had severe cardiovascular defects characterized by local hemorrhages and a swollen pericardial sac.¹⁰⁰ Development of the atria and ventricles is arrested by embryonic day 10.5 and disorganization of the myocardial wall and ventricular atrophy is observed.¹⁰⁰ Subsequent studies using synthesized small-molecules that competitively inhibit the recognition of N-degrons have been performed to determine the specific effects on the heart.¹⁰³ In both mouse and rat cardiomyocytes, these studies demonstrate that the N-end rule pathway functions to regulate cardiac proliferation and hypertrophy, which further supports a role for degrons in cardiac development and the degradation of important cardiac regulators.¹⁰³

The role of Ubiquitin Ligases in Cardiac Disease

Approximately 500 ubiquitin ligases are estimated to exist in the human genome. At least 9 have been described in the heart, with 6 of these (MuRF1, MuRF2, MuRF3, MAFBx/atrogen-1, CHIP, MDM2) being mechanistically characterized in cardiac hypertrophy and ischemia/reperfusion injury. In particular, exciting new studies have been published recently implicating cardiac ubiquitin ligases in cardiac atrophy,¹⁰⁴ cardiac metabolism,¹⁰⁵ and cardiac ischemia/reperfusion injury.⁷⁰ Several recent reviews cover the role of these cardiac ubiquitin ligases in detail, including the 3 additional ubiquitin ligases (Cbl, E6AP, cIAP) that have distinct functions described in other model systems,^{63,64,106–108} and so this topic is not explored further in this review.

The Role of Ubiquitin in Selective Autophagy Ubiquitination, the Proteasome, and Autophagy

Misfolding is a constant threat to proteins and results from the presence of oxidative stress, mutations, and external stresses such as heat shock. To protect against these stresses, the cell has constitutive and inducible molecular chaperones of the heat shock family to assist in refolding. Misfolded proteins have exposed hydrophobic residues that, when properly folded, are not present. Heat shock proteins bind these hydrophobic residues in misfolded proteins and help to refold them (Figure 3A). The first

option of a cell when encountering misfolded proteins is to refold them. However, if refolding is not possible, the protein is slated for degradation by the proteasome. Evidence demonstrates that ubiquitin ligases interact with heat shock proteins, illustrating the close relationship within the cell of the processes involved in monitoring protein quality. These heat shock protein-E3 complexes promote folding and, when this is not possible, enhance ubiquitination of recognized substrates, targeting misfolded proteins for proteasome degradation.¹⁰⁹

Misfolded proteins unable to be resolved by chaperone-mediated refolding or proteasome degradation form aggregates. Aggregates of misfolded proteins may then polymerize to form structures microscopically recognized as inclusion bodies (Figure 3B) and aggresomes (Figure 3C).^{110,111} From these structures, bulky, misfolded proteins can be degraded via pathways that are independent from the proteasome degradation pathway. By shunting protein degradation to these alternative pathways, the accumulation of ubiquitinated misfolded proteins can be prevented. The accumulation of protein aggregates is a proximal trigger of cardiomyocyte autophagy, which is the mechanism by which aggresomes can be cleared.¹¹²

Autophagy occurs continuously at low levels in the normal heart. It is regulated by autophagy (Atg) proteins that make up 2 conjugation pathways that parallel the ubiquitin ligation pathway described in Figure 1: (1) the Atg12-Atg5 pathway; and (2) the LC3(Atg8)-PE (light chain 3-phosphatidylethanolamine) pathways (recently reviewed by Gustafsson and Gottlieb¹¹³). In this system, Atg12/Atg5 or Atg8 (LC3) are conjugated to Atg7, Atg10, Atg5, or Atg3 via lysine residues, forming complexes essential for the recruitment of LC3 and the formation of the membranes needed to form the autophagosomes (Figure 3D).^{114,115} During cardiac ischemia or cardiac loading, autophagy increases as a means to adapt to the significant amount of remodeling that accompanies these processes.^{113,116}

Selective Autophagy Occurs Through Receptors That Recognize Ubiquitinated Proteins

Although the 26S proteasome degrades most intracellular proteins, it is limited in its capacity to degrade misfolded proteins which become aggregated. Because the proteasome is unable to degrade these proteins, parallel systems have evolved to help remove proteins that cannot be degraded by the proteasome. This is done by intracellular receptors which recognize ubiquitinated protein aggregates, and target these proteins for destruction by the autophagosome.^{117,118} Autophagy is a general term to describe several processes in which lysosomes engulf cytosolic proteins for degradation. Recently, a group of loosely associated receptors have been described that recognize ubiquitin chains covalently attached to proteins and are capable of delivering ubiquitinated proteins to autophagosomes. Two of these receptors, p62 and NBR1, target ubiquitinated proteins not cleared by the proteasome for autophagic clearance. This selective uptake of cellular organelles has previously been described for mitochondria, aggregations of protein, and bacteria.¹¹⁹ Identifying the receptors that recognize ubiquitin led to advancements in understanding the underlying mecha-

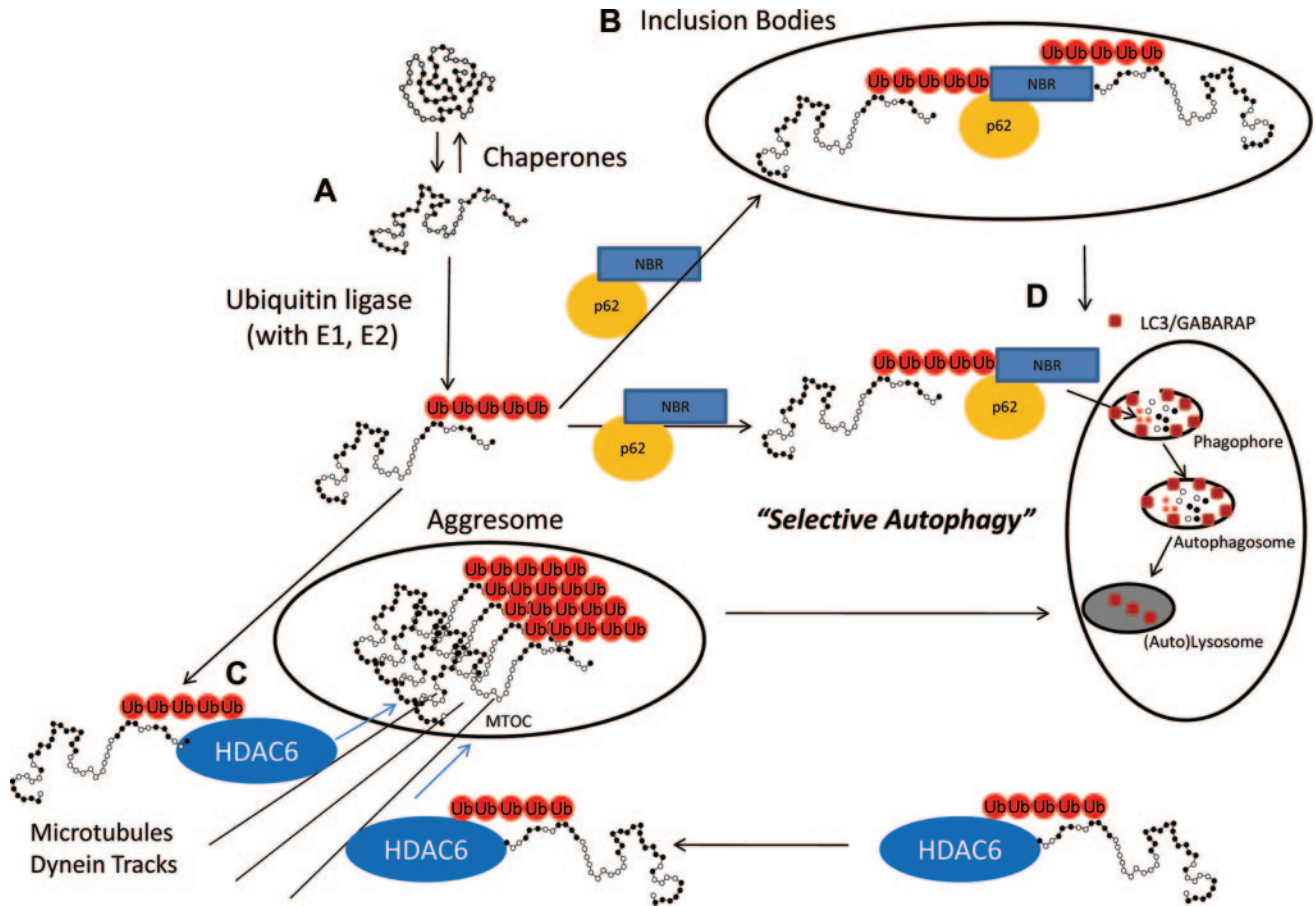


Figure 3. Selective autophagy through recognition of misfolded and ubiquitinated proteins through NBR1 and p62. A, Stress-induced misfolding of proteins is a constant threat to the well being of the cell. Chaperones continually refold proteins by recognizing the hydrophobic regions of the protein exposed during stress. Heat shock protein–ubiquitin ligase complexes promote folding, but if this is not possible, they enhance the ubiquitination of recognized substances effectively targeting proteins for either proteasome degradation (see Figure 1) or by selective autophagy. B and C, Misfolded ubiquitinated proteins can polymerize to form inclusion bodies (B) and aggresomes (C), which form from the transport of aggregated ubiquitinated proteins that are transported via dynein on microtubule tracks. D, “Selective autophagy” is the catabolism of macromolecule and organelles based on the recognition of ubiquitination chains on proteins, which plays an important role in maintaining protein quality control in the cell. MTOC indicates microtubule organizing centers. Adapted from Kirkin et al¹³⁰ and Lamark et al.¹⁴⁴

nisms of the apparently “specific” (selective) autophagy. There is increasing appreciation that ubiquitin plays a role in autophagy and that the uptake of damaged proteins and organelles occurs in a much more specific way than previously realized.

Selective Autophagy: Targeting Ubiquitinated Proteins for Autophagy Through p62, NBR1, Histone Deacetylase 6, and BAG1/BAG3

The multifunctional ubiquitin receptor p62 has recently been identified as part of the autophagic apparatus.¹²⁰ p62 contains a zinc-finger domain and ubiquitin-binding UBA domain in its C-terminal region. The UBA domain is able to bind Lys48-linked and Lys63-linked ubiquitin chains, with higher affinity for Lys63 (Figure 3).^{121–123} The p62-associated clearance of aggregated proteins by autophagy was first suggested by the discovery of colocalization of ubiquitin-positive inclusion bodies with p62.^{124,125} Further evidence for the relationship between p62 and LC3 comes from mouse studies.^{126,127} Mice with deficiencies in

autophagy (Atg7^{-/-} mice) display accumulation of p62 in ubiquitin positive inclusion bodies.¹²⁷ Mice lacking both Atg7 and p62 (Atg7^{-/-}/p62^{-/-} mice) have reduced numbers of protein aggregates.¹²⁷ The role of p62 in the formation of autophagosomes is further supported by similar findings in *Drosophila*.¹²⁸ Recent studies have also found that p62 regulates the clearance of proteins cleared by the UPS. In models in which autophagy is inhibited, p62 accumulation is seen as expected.¹²⁹ Unexpectedly, however, there is also decreased clearance of proteins normally removed by the UPS, such as p53, in addition to the accumulation of aggregation prone proteins.¹²⁹

NBR1 binds ubiquitin by its UBA domain, favoring Lys63-linked poly-ubiquitinated chains.¹³⁰ The recruitment of ubiquitin-linked cargo to lysosomes is dependent on both p62 and NBR1. The cross-linking of ubiquitinated misfolded proteins is mediated by NBR1, and like p62, is necessary for protein aggregation and inclusion body formation following autophagy inhibition.¹³⁰ NBR1 associates with itself through its coiled-coiled domain to clear ubiquitinated misfolded proteins, or it can interact with

oligomeric p62 and ubiquitinated mis-folded proteins.¹³⁰ In muscle cells, NBR1 interacts directly with p62 and has been implicated as a part of a signaling complex of the giant protein titin kinase, where mechanical stretch-inducing titin kinase activity is associated with the regulation of the ubiquitin ligase MuRF2.¹³¹ An additional role for NBR1 in autophagic degradation of ubiquitinated targets is illustrated by the ability of NBR1 to bind directly to the autophagosome-specific ATG8/LC3/GABARP in the presence or absence of p62 (Figure 3D).¹³⁰

Histone deacetylase (HDAC)6 is another adaptor protein that recognizes ubiquitinated and misfolded proteins, shuttling them into aggresomes where they are sequestered within the cell.^{132–134} The aggresomes are then targeted for degradation by the autophagic pathway after HDAC delivers them via the microtubule organizing centers (Figure 3C). When aggresomes are experimentally induced with ubiquitinated proteins, they contain HDAC6. Inhibition of HDAC6 using siRNA reportedly compromises aggresome formation, a process that can be rescued with HDAC constructs containing the ubiquitin binding region.¹³⁵ Like NBR1, HDAC6 binds Lys63-linked ubiquitin chains.¹³³ HDAC6 interacts with dynein motors necessary for the transport of the aggresome via the microtubules (Figure 3C).¹³⁵

Other proteins that do not have ubiquitin binding domains can be associated with ubiquitinated proteins and autophagosomal markers, including BAG1 and BAG3. The Hsc/Hsp70 cochaperone BAG3 has been proposed to mediate autophagic degradation of ubiquitinated proteins in aging cells.^{135–137} BAG3 colocalizes with p62-positive aggregated proteins, but is not itself degraded by autophagy.¹³⁶ In contrast to BAG3 (found primarily in older cells), BAG1 mediates the proteasome-dependent degradation of ubiquitinated proteins in coordination with the CHIP ubiquitin ligase.^{136,138} These findings suggest that the ratio of BAG1/BAG3 changes the mechanism by which aging cells regulate how ubiquitinated proteins are disposed. The activation of the senescence program, including the increase in the BAG3/BAG1 ratio, enhances the shuttling of ubiquitinated proteins to autophagy pathways with advancing age, whereas younger cells with the reverse ratio tend to shuttle ubiquitinated proteins for proteasomal degradation.¹³⁶

Ubiquitination may play a role in selectively degrading whole organelles, such as the mitochondria,¹³⁹ peroxisomes,¹⁴⁰ ribosomes,¹⁴¹ and bacteria.¹⁴⁰ This is most readily seen in the starvation response. The role of ubiquitin in clearing specific organelles is not fully understood. Mitochondrial degradation has been reported to be ubiquitin-dependent,¹⁴² whereas autophagy of the peroxisomes is partially dependent on p62 and associated with mono-ubiquitinated proteins.¹⁴³ There is much work to be done to understand how complex organelles are targeted for selective autophagy through ubiquitination and recognition by adaptor proteins such as p62 and NBR1.

Conclusion

A picture of the UPS as a simple isolated system no longer adequately describes the myriad of functions it has in the

cardiovascular system. The UPS plays a fundamental role in the development of the vascular system through its regulation of key signaling pathways including Notch, VEGF, and HIF1. In the mature vasculature, the UPS regulates inflammation, oxidative stress, and apoptosis in addition to cholesterol metabolism in ways that may affect the development and severity of atherosclerosis. In the stressed heart, the UPS maintains protein quality control of nascent proteins in the ER by the UPR (including signaling through PERK, ATF6, IRE-1) and ER-associated degradation of misfolded proteins. Despite increasing appreciation of the UPS in regulating a myriad of biological processes, some of the newest and most fascinating findings implicate autophagy (“selective autophagy”) as an alternative to the proteasome to get rid of unfolded, damaged, and aggregated proteins. The cytosolic receptors p62, NBR, and HDAC6 play a fundamental role in targeting damaged proteins to this extraproteasomal “selective autophagy” form of destruction. This emphasizes the fundamental role of the UPS in maintaining protein quality by the selective destruction of worn and damaged proteins. Although our appreciation of the UPS in maintaining protein quality control continues to grow, so does our realization of the ubiquitous nature of its control over cell signaling pathways by harnessing the same destructive power.

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Disclosures

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