Circulation

CLINICAL IMPLICATIONS OF BASIC RESEARCH

# Protein Misfolding in Cardiac Disease

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Although mortality from some types of cardiovascular disease is decreasing, future therapies aimed at treating underlying mechanisms may improve clinical outcomes yet further. Based on recent discoveries, it is becoming apparent that defects in intracellular protein quality and age-related impairments in protein homeostasis, or proteostasis, are contributing factors in many forms of cardiac disease. In the heart, such impairments can lead to cardiac myocyte dysfunction and eventually cell death, a particularly important problem given that cardiac muscle exhibits limited regenerative potential. Now, exciting efforts are focused on developing therapies targeting defective proteostasis, some of which have transitioned from preclinical proof-of-concept studies into clinical practice.

## WHAT IS PROTEOSTASIS, WHAT KEEPS IT BALANCED, AND WHY IS THIS BALANCE IMPORTANT?

Protein quality can be compromised by genetically encoded mutations, and by mistakes in transcription or translation, resulting in errors as small as 1 amino acid substitution. This, in turn, can disrupt normal protein folding, intracellular translocation, or function. In addition, disease-related stresses, such as ischemia, can trigger protein misfolding, and unfolding of proteins that were once properly folded and functional, as well. Exposed hydrophobic regions of misfolded proteins bind to similar regions in nearby proteins, leading to inappropriate associations and protein aggregation. The aggregating species can continue to bind exposed hydrophobic regions of neighboring proteins and thus have the potential to form stable, both soluble and insoluble, nonfunctional structures.

These protein aggregates interfere with normal cell function in numerous ways, including by disrupting protein complexes, interacting with lipids and membranes, and possibly interfering with contractile function. Protein aggregates can recruit and sequester components of the proteostasis machinery, including chaperones and elements of the ubiquitin and proteasome machinery, presumably to aid in the clearance of the aggregated species, facilitating dysregulated proteostasis and chronic activation of stress responses, which can result in cell death.[1] Moreover, whereas most misfolded proteins are prevented from transiting out of the cell, some can be secreted from their cell of origin and deposited in distant tissues. This results in deleterious functional consequences deriving from pathogenic proteins clustering on plasma membranes, altering lipid bilayer fluidity, membrane architecture, and the normal distribution of membrane proteins.

A central tenet of cell biology is that protein synthesis takes place where the ribosomes are located. This means that, in eukaryotic cells, protein synthesis occurs in the cytoplasm, and on the ribosome-studded rough endoplasmic reticulum (synthesis of secreted and membrane proteins) and in mitochondria, as well (Figure A).



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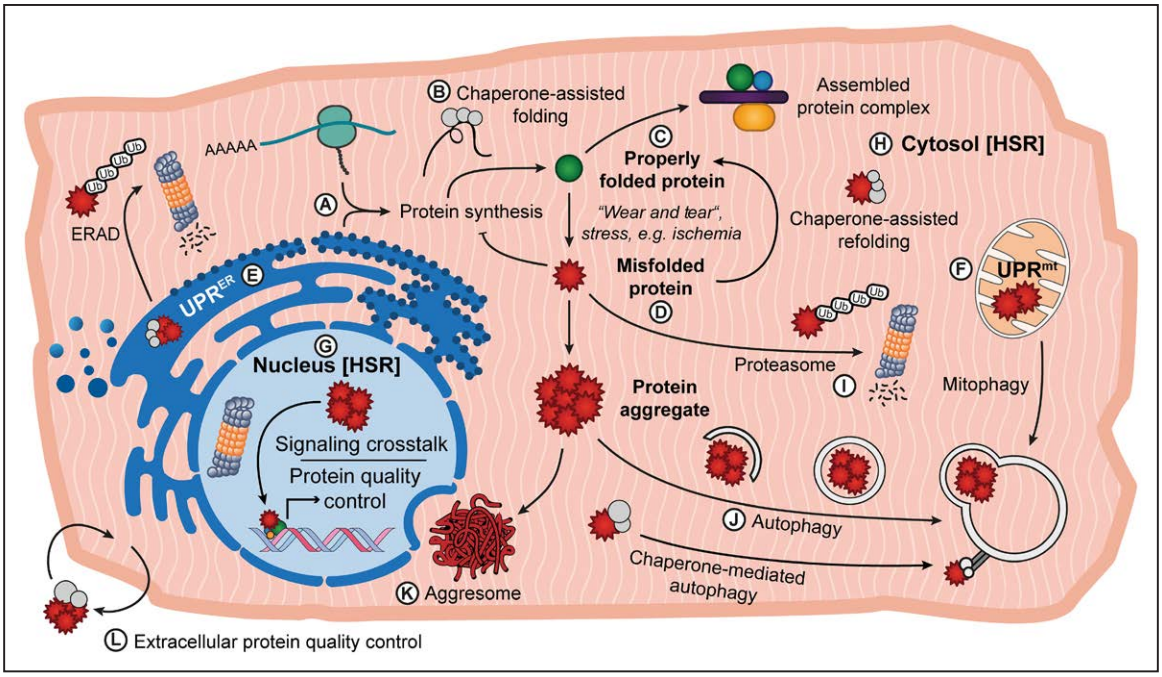


**Figure. Proteostasis checkpoints in cardiac myocytes.**
Proteostasis is maintained by a series of protective mechanisms in various subcellular compartments that can be overwhelmed in the diseased heart. In physiological conditions, protein synthesis at the endoplasmic reticulum (ER), or on free ribosomes (**A**), is aided by chaperones (**B**), is tightly regulated, and results in the production of properly folded, functional proteins (**C**). Conditions of stress, or wear and tear, result in protein misfolding (**D**). Cells have evolved the ER unfolded protein response (UPR), which includes ER-associated protein degradation (ERAD; **E**), as well as mitochondrial UPR (UPR$^{mt}$; **F**), and nuclear (**G**) and several cytoplasmic stress response pathways, including the cytoplasmic heat shock response (HSR; **H**), the ubiquitin-proteasome system (**I**), and autophagy (**J**), aimed at preventing and resolving protein damage, misfolding, and aggregation. Aggregation of misfolded proteins in aggresomes (**K**) happens when protein misfolding is not resolved. In addition to intracellular protein misfolding, extracellular protein aggregates contribute to impaired cardiac function and are cleared by chaperones and proteases (**L**).



To be functionally active, a protein must acquire the proper 3-dimensional conformation, a process orchestrated by molecular chaperones. In fact, protein folding often begins during protein synthesis on ribosomes and depends on pH, redox status, concentrations of salts and ions, and the presence of chaperones and cofactors, all of which can be perturbed in disease-stressed cells (Figure B through D).

Protein misfolding attributable to genetic mutations, cellular stress that damages nascent or aging proteins, or normal wear and tear is detected by the cytosolic or nuclear heat shock responses and the endoplasmic reticulum or mitochondrial unfolded protein responses, all of which initially aim to promote protein folding through upregulation of chaperones and other cofactors (Figure E through H). Given that they impair cell function and can cause cytotoxicity, terminally misfolded proteins are targeted for degradation mainly through the ubiquitin–proteasome or autophagy–lysosomal pathways (Figure I and J). Aggregation of misfolded proteins in aggresomes occurs when protein misfolding is not resolved (Figure K). A handful of chaperones can accompany proteins to the extracellular space where they can fortify protein folding, and proteases in the extracellular space contribute to the degradation of misfolded proteins (Figure L). It is now clear that the functionality of these regulatory proteostasis checkpoints is challenged during aging, promoting age-related decreases in the capacity of cells to maintain proteostasis, rendering the organism susceptible to aggregation events that are hallmarks of age-associated proteinopathies, best studied in neurodegenerative disorders such as Alzheimer's and Parkinson's disease, but recently also described in the aging heart.[2]

## HOW DO ALTERATIONS IN CARDIAC PROTEOSTASIS CONTRIBUTE TO OUR UNDERSTANDING OF THE CAUSE AND PROGRESSION OF HEART DISEASE?

Misfolded proteins in the heart can be of cardiac or extracardiac origin. Among the most overt manifestations of intracardiac accumulation of misfolded proteins is amyloid cardiomyopathy, a disorder in which immunoglobulin light chain protein or transthyretin (wild-type or mutated) deposit in cardiac tissue and provoke heart failure. In the vasculature, defects in proteostasis in the arterial wall are related to atherosclerosis initiation and progression. In cardiac tissue, patients with hypertrophic cardiomyopathy, nonischemic cardiomyopathy, or idiopathic dilated cardiomyopathy present with misfolded soluble protein oligomers in cardiac myocytes. Hearts of patients with dilated cardiomyopathy or ischemic heart disease also manifest aberrant protein aggregation and

accumulation of ubiquitylated proteins, and activation of unfolded protein responses, as well. Moreover, desmin-related cardiomyopathy, caused by mutations in desmin or associated proteins, is characterized by accumulation of misfolded proteins that impair cardiac function. Studies in mice have also shown that pressure overload and ischemic stress impair cardiac proteostasis and contribute to contractile dysfunction.

Mouse models of protein aggregate formation in the heart manifest cardiac myocyte death and heart failure. In most of these studies, it remains unknown which forms of abnormal proteins (eg, soluble or insoluble) are toxic. Moreover, whether protein aggregates are the trigger or the consequence of a given disease process varies with the disease. For example, depletion of oxygen and ATP during ischemia elicits protein misfolding, whereas, in the setting of hypertrophic cardiomyopathy, normal cellular folding capacity can be overwhelmed by the increased demand; in the complex setting of heart failure, dysregulation of energy metabolism, cellular redox status, calcium homeostasis, and glycosylation disrupt proper folding of proteins. For additional details, readers are referred to a comprehensive review by Henning and Brundel.[2]

## HOW CAN WE EFFECTIVELY DIAGNOSE ALTERATIONS IN CARDIAC PROTEOSTASIS?

Detection of misfolded proteins in tissues is the first step in the diagnosis of proteinopathies. A wide range of approaches is currently available, including noninvasive strategies such as cardiac MRI, scintigraphy with radiolabeled phosphate derivatives, positron emission tomography, and more. The gold standard remains evaluation of tissue derived from endomyocardial biopsy followed by the use of histological dyes specific for protein aggregates. Readers are referred to recent comprehensive reviews.[3,4]

## WHAT ARE PROTEOSTASIS-BASED THERAPEUTIC STRATEGIES TO CONSIDER FOR CARDIAC DISEASE?

The development of therapeutics directed at proteostasis impairment in heart disease is just beginning. For example, small-molecule activators of the heat shock response, such as geranylgeranylacetone, are used in Japan for treatment of ulcers, and an endoplasmic reticulum unfolded protein response activator, such as the ATF6 pathway activator compound 147, is protective in myocardial, renal, and cerebral ischemia/reperfusion models.[5] This strategy to activate stress response pathways and chaperone activity establishes a cytoprotective state against misfolding and aggregation, and represents a promising therapeutic approach.

Alternatively, therapeutic approaches that focus on decreasing misfolded protein concentrations may be effective in cardiac disorders in which deposits accumulate slowly over time. In this regard, enhancement of protein clearance mechanisms either by activation of autophagy (eg, rapamycin or spermidine) or by pharmacological proteasome induction may be promising. Furthermore, strategies that reduce protein synthesis to inhibit further protein misfolding, or use of chemical chaperones such as 4-phenylbutyrate, have met with success in preclinical models.

As an exciting development, novel targeted therapies have emerged with clinically meaningful benefit in amyloidosis. Patisiran, the first small interfering RNA–based therapeutic approved by the US Food and Drug Administration, specifically blocks transthyretin synthesis, improving functional outcome in patients with hereditary transthyretin amyloid cardiomyopathy.[4] Tafamidis acts as a chaperone to stabilize transthyretin in the correctly folded, tetrameric conformation, reducing mortality and cardiovascular hospitalizations and improving functional capacity and quality of life in patients with transthyretin amyloid cardiomyopathy.[4]

Genetic testing can be used in at-risk populations, such as individuals of African ancestry. The most common amyloidogenic transthyretin variant, Val122Ile, which is associated with a predominantly cardiac phenotype with late onset, is present in 3% to 4% of African Americans.[4] Moreover, a study of patients with heart failure with preserved ejection fraction reported that wild-type transthyretin amyloidosis accounts for a significant number (13%) of heart failure with preserved ejection fraction cases. Indeed, it is widely believed that cardiac amyloid is underdiagnosed, and enhanced clinical awareness is emerging rapidly.

Lurking underneath the excitement regarding amyloid disease, alterations in protein homeostasis, in general, are emerging as relevant therapeutic targets in cardiovascular disease. The clinical implications of this rapidly evolving basic science are robust and herald therapeutic relevance in coming years.

## ARTICLE INFORMATION

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## Disclosures

None.

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