Biological and Chemical Approaches to Diseases of Proteostasis Deficiency

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Abstract

Many diseases appear to be caused by the misregulation of protein maintenance. Such diseases of protein homeostasis, or "proteostasis," include loss-of-function diseases (cystic fibrosis) and gain-of-toxic-function diseases (Alzheimer's, Parkinson's, and Huntington's disease). Proteostasis is maintained by the proteostasis network, which comprises pathways that control protein synthesis, folding, trafficking, aggregation, disaggregation, and degradation. The decreased ability of the proteostasis network to cope with inherited misfolding-prone proteins, aging, and/or metabolic/environmental stress appears to trigger or exacerbate proteostasis diseases. Herein, we review recent evidence supporting the principle that proteostasis is influenced both by an adjustable proteostasis network capacity and protein folding energetics, which together determine the balance between folding efficiency, misfolding, protein degradation, and aggregation. We review how small molecules can enhance proteostasis by binding to and stabilizing specific proteins (pharmacologic chaperones) or by increasing the proteostasis network capacity (proteostasis regulators). We propose that such therapeutic strategies, including combination therapies, represent a new approach for treating a range of diverse human maladies.

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INTRODUCTION

Understanding how the unfolded ensemble of polypeptides resulting from the process of translation arrives at their native structure(s) for function and how these structures are maintained and ultimately turned over is a major challenge. We need to understand how the cell interprets protein energetics and influences protein folding in both human health and disease. Unlike protein folding in the test tube, a protein in a eukaryotic cell must fold and function in a crowded environment, as well as in a variety of distinct environments defined by the cell's compartmentalized organization, e.g., the cytoplasm, exocytic and endocytic compartments, the mitochondria, the nucleus, and the extracellular space. Moreover, proteins are subject to extensive changes in structure as they cycle between inactive and active conformations in response to posttranslational modification(s) and/or as they engage in the protein-protein interactions that enable their biology.

Proteins face many challenges to normal folding, refolding, and function owing to a constant barrage of physical, metabolic, and environmental stresses. These include changes in the concentration and composition of small-molecule metabolites that strongly influence folding by binding-induced modulation of protein stability, changes in the concentration of osmolytes that influence protein stability through modulating the hydrophobic effect and hydrogen bonding, changes in temperature that influence energetics, and changes in the concentration of reactive oxygen species (ROS) or oxidized small molecules that result from reactions with ROS that alter the structure and function of proteins by aberrantly modifying them. These modifications can denature the folded protein to render it inactive and/or trigger folded or natively unstructured proteins to aggregate and become toxic to the cell.

We now recognize that the chemical and energetic properties specified by the amino acid sequence of each polypeptide (the primary structure) encoded by the genome, while very important in partially determining the folding energy landscape, are only part of how proteins evolve biological function. Numerous macromolecular assistants exist in cells to influence the folding of the proteome. There is also an

intrinsically unstructured proteome that, owing to the sequences of its components, probably never adopts a permanent three-dimensional structure but likely requires macromolecular assistance to prevent aggregation and promote function. In the case of the folded proteome, such assistance controls the rate of protein synthesis; influences the rate of folding of the proteome; effects membrane trafficking patterns responsible for compartmental localization, which influences stability; and mitigates aggregation and mediates degradation, enabling protein turnover. These competing biological pathways comprising hundreds of components make up the proteostasis (protein homeostasis) network (1).

The proteostasis network and its pathways are controlled by numerous integrated signaling pathways. These signaling pathways, a subset of which are stress responsive, including those that respond to misfolding proteins, optimize the capacity of the proteostasis network to maintain protein function. This occurs by more efficiently folding the natively folded proteome and maintaining the natively unstructured proteome in a nonaggregated state in response to challenges from the inherited proteome (e.g., mutated misfolding-prone proteins), somatic mutations that result in loss of function or disruption of proteostasis capacity (e.g., due to misfolding or aggregation), and the environment (including oxidative stress). The proteostasis network is present in all extant life and is therefore ancient. It necessarily coevolved with the remarkable diversity of polypeptide sequences to enable the evolution of a wide variety of organisms, by expanding the capacity of proteins to function in increasingly complex cellular and subcellular environments, and to perform increasingly specialized cellular tasks.

When a protein fails to fold properly owing to an alteration in its sequence (e.g., a mutation or an aberrant posttranslational modification caused by oxidative stress), and/or possibly as a consequence of a change in the concentration, and/or the composition of the components of the proteostasis network, there is a breakdown in biology. Loss-of-protein function or gain-of-toxic function (the latter often being associated with aggregation) can trigger disease by interfering with cell function (2–4). The multiple roles of the proteostasis network in maintaining the proteome and, therefore, normal physiology in response to challenges during development, aging, and stress are only beginning to be appreciated (1).

To understand the operation of the proteostasis network in health and disease, we present a hypothesis about biologically assisted folding that integrates folding energetics. Through mathematical modeling, wherein folding energetics and proteostasis network component composition and concentration are variables, we propose a framework for how the proteostasis network interprets and influences protein folding energetics to control the efficiency of folding. We introduce the notion of a minimal "proteostasis boundary," that is, the folding energetics required to achieve folding of a protein at a given proteostasis network capacity. Using modeling approaches, we illustrate that, although the proteostasis network can make up for deficiencies in folding energetics and is thus remarkably adaptable, there are limits set by the folding energetics. We build on the proteostasis boundary hypothesis to illustrate what can go wrong in proteostasis diseases. We review the demonstrated biological and chemical strategies to ameliorate loss- and gain-of-function misfolding maladies by adjusting the stability of the fold or the capacity of the proteostasis network, or both, leading us to propose a view of how enhanced proteome maintenance can be used to ameliorate numerous diseases of complex etiology facing humanity in the twenty-first century.

PROTEIN FOLDING IN VITRO

The challenges associated with transforming a largely unordered ensemble of conformations resulting from translation (the unfolded state) into a few closely related three-dimensional

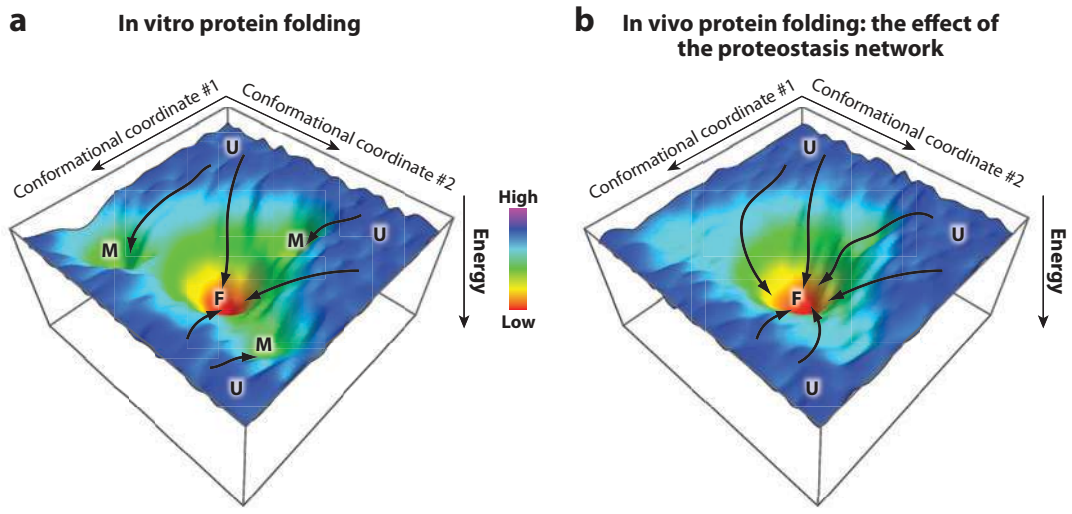

Figure 1

The folding energy landscape for a hypothetical polypeptide. The major conformational ensembles are labeled U (unfolded state), M (misfolded state), and F (folded state). (*a*) A given energy landscape in vitro. (*b*) The same energy landscape in vivo influenced by the proteostasis network, which is envisioned to minimize the aggregated/misfolded population, thereby improving folding efficiency.

structures that enable function (the folded state) is as ancient as life itself. In its simplest form, it can be described by the fundamental principles put forward by Anfinsen (5) that the native or folded state of a protein is the most thermodynamically stable structural ensemble—one that is determined by the amino acid sequence and the solution conditions. A contemporary view of the energetic component of protein folding is illustrated by folding energy landscapes (**Figure 1**) (6). Here, the unfolded state, represented by the high-entropy state at the top of the funnel, folds by multiple parallel pathways and sometimes detectable intermediates to achieve its most thermodynamically stable (lowest energy) ensemble of closely related structures, the natively folded state.

Although there are many energetically accessible paths down the folding funnel (**Figure 1**) (7), some with local energy minima that could trap the protein in partially folded, misfolded, and/or aggregated states (**Figure 1*a***), the chemistry and energetics of the polypeptide chain impose constraints that limit the number of pathways that are actually used in a given aqueous solvent and at a given temperature (8). Even largely unordered conformers can have hydrophobic clusters and/or groups of electrostatic interactions that restrict the available pathways a given protein can use to get to the folded state ensemble (9). Folding transition states are often associated with the formation of reverse turns or hydrophobic clusters that act as kinetic "gatekeepers" in acquisition of the folded state (8, 10).

We now appreciate that the folded state is not simply the snapshot obtained by X-ray crystallography; rather, the folded state is a dynamic ensemble of closely related conformers. The extreme extension of this concept is the realization that a fraction of the human proteome is intrinsically disordered and that this high degree of flexibility enables interactions (sometimes facilitated by posttranslational modifications) with multiple partners (11). Although the rules directing the physical chemistry of protein folding remain to be fully elucidated, rapid advances in this area now allow us to predict with increasing accuracy the folds of small proteins (<100 kDa) based solely on the chemical and physical properties of the polypeptide chain (8, 12).

BIOLOGICALLY ASSISTED PROTEIN FOLDING

Although small, single-domain proteins fold with amazing speed and efficiency at low concentrations in aqueous buffers, achieving and maintaining the folds of larger, multidomain soluble or membrane proteins is far more challenging, if not impossible in vitro. The folding of soluble and membrane-associated multidomain proteins is also challenging in vivo, as highlighted below.

The rate of folding in a cell is affected by the highly regulated rate of translation and, therefore, by several factors including the composition of the translation components, including aminoacyl tRNA pools. Cellular folding kinetics are also affected by both synonymous (single nucleotide polymorphisms that alter the nucleic acid but not the amino acid sequence) and nonsynonymous (nonsense and missense mutations that change the amino acid sequence) substitutions in the genome that alter the rate of translation and, in the case of the latter, the sequence of the nascent chain (13, 14).

The environment of the cell is very crowded (15) and, therefore, has high protein concentrations that promote aggregation. Cellular protein aggregation is associated with proteotoxicity and must be minimized, especially during chemical, physical, and metabolic stress. The cellular folding of multidomain proteins is generally slow and involves significant populations of folding intermediates. This is especially true for transmembrane proteins that must fold in the context of multiple local environments: the lipid bilayer for the transmembrane domains and the aqueous environment for the lumenal and cytoplasmic domains (16).

Protein folding, unfolding, and refolding are constantly occuring throughout the lifetime of nearly all proteins. Thus, the fold is highly dynamic and must be protected as it proceeds through rapid changes in conformation, simply as a consequence of its folding equilibrium or in response to posttranslational modifications and/or interactions with a variety of protein partners required for its function (11).

Finally, proteins have distinct turnover rates that are integrated with function, and this fine-tuning can become defective, contributing to disease. Protein turnover rates are generally linked to the life span of the cell, suggesting that this is an adaptable feature of biologically assisted folding.

THE PROTEOSTASIS NETWORK

It is the job of the cellular proteostasis network to enable cellular protein folding and function in the face of all of the challenges discussed above. The proteostasis network consists of numerous biological pathways. The macromolecular components of these pathways comprise over 1000 general and specialized chaperones, folding enzymes, and degradation components as well as trafficking components, the latter influencing compartmental localization. Control of the proteostasis network is accomplished by signaling pathways that directly regulate the concentration, distribution, and activities of the components that make up the proteostasis network and, therefore, the relative activities of the biological pathways in the network (17, 18) (**Figure 2**).

The folding function of the proteostasis network is accomplished by smoothing the energy landscape of proteins (**Figure 1*b***) using chaperones and folding enzymes which bind to intermediates and transition states, respectively. Chaperones promote folding and maintenance within the cell largely by minimizing misfolding and aggregation, and chaperones are specialized for various compartments of the cell. Different cells have varying proteostasis capacities reflected in the composition and concentrations of their proteostasis components, presumbly to handle the different folding challenges that arise in response to differentiation during development. Cytosolic chaperones include the ribosome-associated chaperones, the Hsp40/Hsp70 and Hsp90 chaperone systems, and the chaperonins, such as TRiC, that facilitate folding by encapsulation (19–21). There are analogous Hsp40/Hsp70/Hsp90 cognate chaperones found in the endoplasmic reticulum

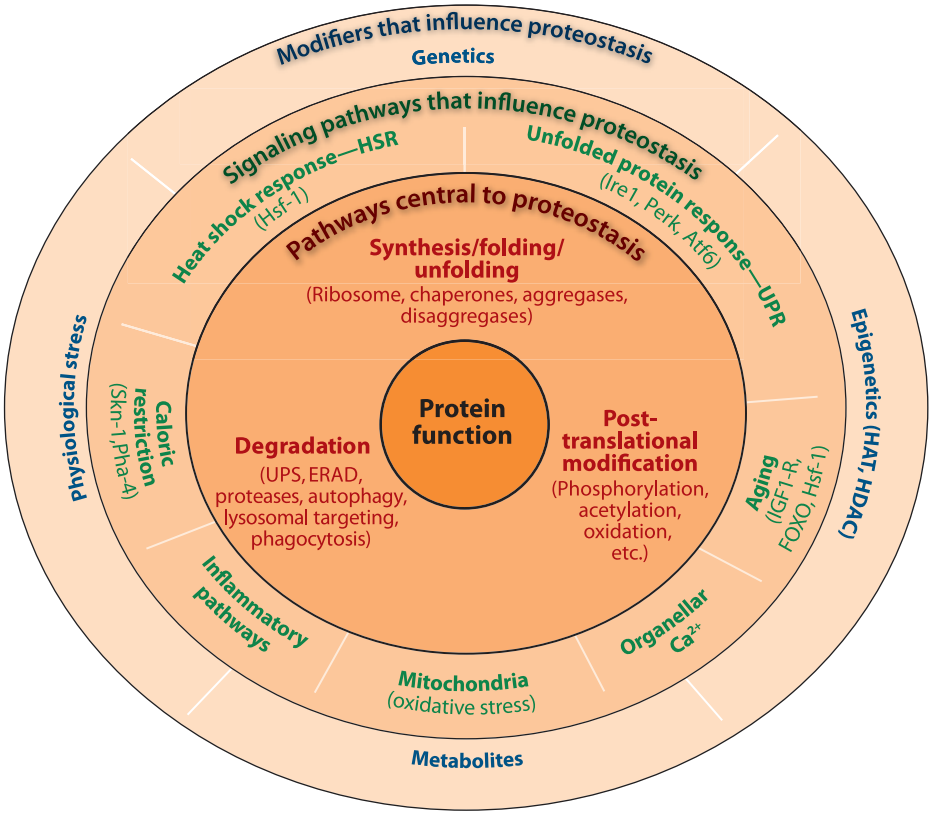

Figure 2

Managing proteostasis. Illustrated are the layers of interactions that facilitate the function of the proteostasis network to generate and maintain functional proteins. The proteostasis network is composed of the components outlined in the first layer (in *red font*), including the ribosome, chaperones, aggregases, and disaggregases that direct folding, as well as pathways that select proteins for degradation [e.g., the ubiquitin-proteasome system (UPS), endoplasmic reticulum (ER)-associated degradation (ERAD) systems, proteases, autophagic pathways, lysosomal/endosomal targeting pathways, and phagocytic pathways, the latter are responsible for the recognition, uptake, and degradation of extracellular proteins]. The second layer includes signaling pathways (in *green font*) that influence the activity of components found in the first layer. The third layer (in *blue font*) includes genetic and epigenetic pathways, physiologic stressors, and intracellular metabolites that affect the activities defined by the second and first layers.

(ER), as well as a number of compartment-specific folding specialists (22, 23). Chaperones collaborate with folding enzymes, including redox enzymes that promote oxidative folding (disulfide bond formation) in the ER (24) and peptidyl-prolyl isomerases that catalyze *cis-trans* amide bond isomerizations in protein folding. Many chaperones specializing in the folding of a given protein or a group of related proteins exist, including, for example, Hsp47 for collagen (25) and microsomal triglyceride transfer protein (MTP) for apolipoprotein B-containing chylomicron particles (26). On the basis of our current understanding, chaperones are best thought of as macromolecules that bind to exposed hydrophobic surfaces in

misfolded or aggregated states and, in a nucleotide hydrolysis-dependent fashion, change conformations affording the previously bound protein another opportunity to fold (27–29). Chaperones and folding enzymes facilitate the folding of multidomain proteins, likely through transient sequestration of folding intermediates, enabling putatively well-choreographed events that would otherwise be difficult to achieve on a biological timescale in the complex environment of the cell. Strong evidence for an extracellular chaperone system is currently lacking, although it is conceivable that the immune system recognizes and clears misfolded extracellular proteins in the context of the proteostasis program. Numerous factors influence the properties of the proteostasis network including changes in cellular ATP levels, amino acid pools, metabolites, lipid homeostasis, and ion balance. These not only alter folding capacity, but also modulate the activity of a number of other proteostasis network pathways such as degradative pathways, including ubiquitin-based proteasome-mediated pathways, and lysosomal and autophagic trafficking pathways (**Figure 2**) that are integral to maintenance of proteostasis (30–33).

Signaling pathways that regulate protein synthesis, folding, trafficking, aggregation, disaggregation, and degradative pathways of the proteostasis network include: the unfolded protein response (UPR), which principally influences ER folding capacity (34–36); the heat shock response (HSR), which balances proteostasis capacity with demand in the cytosol (17, 37); pathways that influence subcellular Ca^{2+} concentrations, such as ER Ca^{2+} concentrations, which can increase the ability of the cell to handle the folding of N-linked glycoproteins in the ER (about one third of the human proteome) via Ca^{2+}-sensitive folding chaperones (calnexin, calreticulin, and BiP) (38, 39); inflammatory responses, which regulate cell defense and death pathways (40, 41); and histone deacetylase (HDAC) pathways, which may integrate proteostasis capacity through epigenetic pathways (**Figure 2**). Such signaling circuits control the capacity and composition of the proteostasis network through transcriptional, translational, and posttranslational mechanisms to balance or rebalance proteostasis by reducing demand, enhancing folding and repair processes such as disaggregation, and/or by mediating degradation. When proteostasis is severely compromised, as can occur in some early- and late-onset genetic diseases associated with the inheritance of a misfolding-prone protein, unsuccessful signaling attempts to rebalance proteostasis can activate cell death pathways, targeting the cell for destruction (34–36). In multicellular organisms, proteostasis pathways can be under cell-nonautonomous global control by neuronal and possibly nonneuronal signaling pathways (42, 43).

The proteostasis network is not only highly adaptable, as discussed above (enabled by the influence of multiple stress-responsive signaling pathways), but it also can be quite distinct in each cell type; see **Figure 3** and **Supplemental Figure 1** (follow the **Supplemental Material** link from the Annual Reviews home page at **http://www.annualreviews.org**) (44). This is not surprising given the central role of proteins in cell physiology and the diversity of function(s) in distinct cells entrained by developmental pathways. The response to diversification includes proteostasis network components that are conserved throughout evolution (42) and those that are specialized (25, 26), reflecting the many different proteostasis challenges. For example, hepatocytes, plasma cells, and β-cells must produce high levels of distinct secreted proteins, whereas a fibroblast has less secretory activity and, therefore, a less-specialized ER proteostasis network capacity. Indeed, the compositional complexity of the proteostasis network scales with the complexity of the organism, consistent with the hypothesis that the proteostasis network influences the evolution of protein sequences. In other words, a protein's ability to achieve its functional state (which, in part, directs phenotypic selection) is dependent on the proteostasis network. Thus, we suggest that any effort to understand protein folding, and therefore protein function, in vivo will ultimately need to consider the

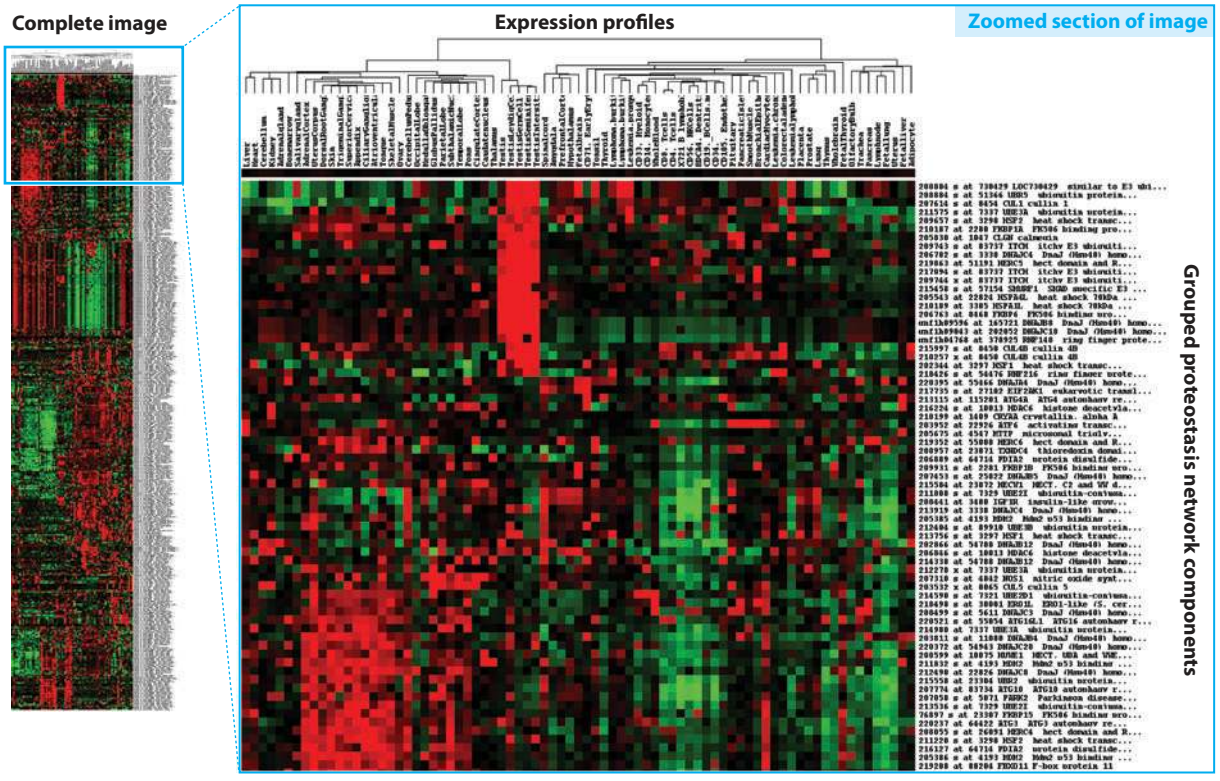

Figure 3

Expression profiling of human proteostasis network components in 80 human tissues (44). Hierarchical clustering was used to group proteostasis network components (y-axis on right) on basis of the similarity of their expression profiles across the 80-tissue array (x-axis on top). A dendrogram is provided where lengths of the branches leading up to each node directly reflect the degree of correlation between the expression profiles as assessed by the pair-wise similarity function described previously (171). To see the high-resolution image that can be zoomed to identify the individual components and tissues see **Supplemental Figure 1**. The heat map illustrates abundance as higher (*red*) or lower (*green*) relative to the mean (*black*) value across all tissues.

interdependence of folding energetics and the proteostasis network (1, 8, 12). The degree to which the proteostasis network biases foldability and function remains to be determined, but it is clear that many, if not most, proteins cannot fold without assistance within the cell.

INTEGRATING FOLDING ENERGETICS WITH THE PROTEOSTASIS NETWORK CAPACITY

Although the energy landscape view of protein folding is a useful framework for interpreting experimental folding studies carried out in vitro (**Figure 1*a***), it does not directly illustrate the critical interplay between the energetics of the protein (defined by the dynamics of the populations of the unfolded, intermediate, and folded states) and the biology of folding. Qualitatively, we know that chaperones and folding enzymes bind to folding intermediates and transition states, resculpting the folding free-energy landscape (**Figure 1*b***). While useful, it does not inform us on how the proteostasis network maintains proteostasis.

One semiquantitative approach toward understanding the interdependence of protein folding energetics and biological folding influenced by proteostasis network capacity has been developed using mathematical modeling

(45, 46). Specifically, we have modeled aspects of the proteostasis network to show how different levels of proteostasis network capacity handle the folding and trafficking of proteins in the exocytic pathway as a function of kinetic and thermodynamic parameters. The FoldEx model (46) describes how the inherent energetics of the polypeptide chain are interpreted by and then influenced by the proteostasis network within the ER, a compartment that plays a central role in the biogenesis and export of proteins to multiple cellular compartments and the cell surface (**Figure 4*a***). In this model, translation, chaperoning, export, and degradation pathways were each treated as single entities, using a Michaelis-Menten kinetic formalism generally reserved for studying enzyme kinetics (**Figure 4*a***). The mathematical modeling enabled us to predict how distinct secretory protein energetics can be influenced by differing proteostasis network activities. The FoldEx model revealed that the energetics of the polypeptide chain and the capacity of individual pathways of the proteostasis network together determine the extent to which a destabilized protein will fold and be exported or be degraded (**Figure 4*a***) (46). Importantly, this model can qualitatively fit experimental data, suggesting that, even with its simplifications, it is able to rationalize and predict experimental observations. The FoldEx model was used to define a "minimal export threshold," a boundary in the three-dimensional space defined by protein stability, folding rate, and misfolding rate. The location of this boundary depends on the proteostasis network capacity, i.e., the concentration and activities of the translation, chaperoning, export, and degradation pathways of the proteostasis network and the protein folding and misfolding energetics. Proteins with energetics within the export threshold at a defined proteostasis network capacity are exported at levels sufficient for function; those with energetics outside the boundary are not.

We want to emphasize that the position of the minimal export threshold, defined by the variables in the FoldEx model, is strongly influenced by the capacity of the proteostasis network to generate and maintain the folded state of a protein in the ER for export. Consequently, whether a protein is exported efficiently enough for it to function depends on both protein folding energetics and the proteostasis network capacity. Although simplified, the Michaelis-Menten treatment of these interactions revealed that there is no single parameter, such as the thermodynamic stability, the folding rate, or the misfolding rate, that determines whether a given protein will fold, traffic, and generate sufficient activity in its destination environment to enable function. Thus, the FoldEx model provides a useful theoretical framework that semi-quantitatively recapitulates experimental data to rationalize the general operation of the exocytic pathway in the context of folding energetics and the adjustable proteostasis network capacity (46).

THE PROTEOSTASIS BOUNDARY

The basic understanding of the function of the ER revealed by the FoldEx model should also be useful for understanding the interplay between the chemistry and the biology of protein folding in other cellular compartments, each with distinct environments and proteostasis network composition and capacity. Other cellular compartments include the many post-ER exocytic and endocytic trafficking compartments, the cytoplasm, the mitochondria, and the nucleus. The concept of the minimal export threshold from the FoldEx model should be extendable to cover any situation in which protein folding energetics and the proteostasis network combine to determine whether a protein can achieve adequate levels of folding for function. We refer to this more general mathematical model as Folding for the Function of protein x or FoldFx (**Figure 4*b***). FoldFx, like FoldEx, enables the interdependence of folding energetics and the proteostasis network capacity to be visualized. As in FoldEx, we treat the translation machinery (**T** in **Figure 4*b***), chaperones (**C** in **Figure 4*b***) and degradation pathways (**D** in **Figure 4*b***) as single entities. The export process

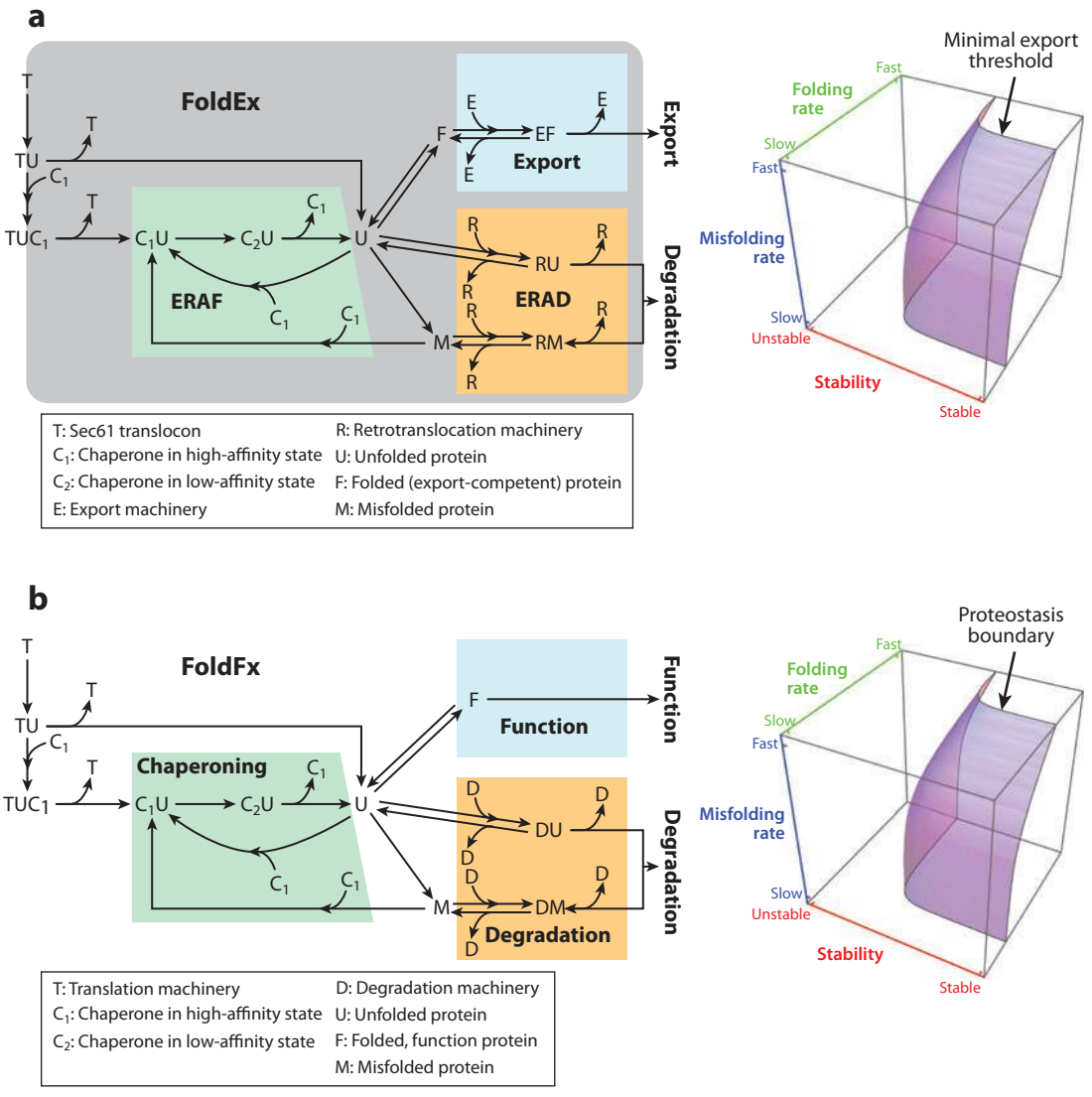

Figure 4

Models for proteostasis. (*a*) (*left*) The FoldEx model for protein folding and export from the endoplasmic reticulum (ER) (46). The model includes ER-assisted folding (ERAF), ER-associated degradation (ERAD), and export. (*right*) A plot showing the minimal export threshold as a surface in a space defined by protein thermodynamic stability, folding kinetics, and misfolding kinetics. Proteins with folding energetics within the boundary are exported efficiently enough to ensure adequate function in their destination environment (46). (*b*) (*left*) The FoldFx model for protein folding and function. FoldFx is largely analogous to FoldEx, except that the export step is replaced simply by protein function, and it is not limited to describing protein folding in a single cellular compartment. (*right*) A plot similar to the one in panel *a*, except that it shows the proteostasis boundary instead of the minimal export threshold. Proteins with folding energetics that lie within the proteostasis boundary fold efficiently enough to ensure adequate levels of function.

in FoldEx (**Figure 4*a***) is replaced in the FoldFx model with function (**F** in **Figure 4*b***), which now simply reflects the generation of a functional protein. Thus, in the FoldFx view, translation, chaperones, and degradative pathways are the factors that determine the levels of functional protein by interpreting and influencing folding energetics. Signaling

pathways (e.g., UPR, HSR, Ca^{2+}, etc.) that control the composition and concentration of proteostasis components through transcriptional and posttranslational modifications are accommodated in this simplified view by virtue of the adjustable concentrations of the proteostasis network components (T, C, and D in **Figure 4*b***) (46).

Like the minimal export threshold used to understand the joint role of folding energetics and the proteostasis network capacity in the exocytic pathway in the FoldEx model (**Figure 4*a***) (46), the combined role of energetics and the proteostasis network in folding efficiency in other compartments is best viewed in terms of a minimal proteostasis boundary, which we refer to simply as the proteostasis boundary (**Figure 4*b***). The proteostasis boundary defines the folding energetics that a protein must have to achieve adequate levels of function in the context of a given proteostasis network capacity in a given cell. Like the minimal export threshold in FoldEx, the proteostasis boundary is best illustrated as a boundary in three-dimensional space, defined by protein folding thermodynamics (from unstable to stable—the x-axis), folding kinetics (from slow to fast—the y-axis) and misfolding kinetics (from slow to fast—the z-axis), a convention used throughout this review (**Figure 4*b***). Like the minimal export threshold, the location and shape of the proteostasis boundary is highly dependent on both the composition and concentration of proteostasis network components.

FoldFx simplifies, with rather broad strokes, the interdependence of folding energetics and proteostasis network capacity in generating a population of protein x that is folded and functional. Of course, the composition of the various proteostasis networks, dictating the position of the proteostasis boundary, is expected to be different for each of the different compartments in a cell (e.g., ER, cytosol, mitochondria, nucleus, etc.), reflecting their specialized functions. Moreover, we envision that a specific protein would only require a subset of the proteostasis network components, which in turn suggests that the proteostasis boundary could be different for proteins that utilize a different set of proteostasis network components, all else being constant. Expanding the FoldFx model to encompass the actual complexity of distinct groups of proteins would certainly add much needed detail. However, we believe that it is not necessary to create multiple proteostasis boundaries for individual or groups of proteins comprising the proteome of an organism in order to illustrate how the model can help us understand the role of folding energetics and proteostasis network capacity in health and disease, and the pharmacologic management thereof.

PROGRAMMING THE PROTEOSTASIS BOUNDARY

In **Figure 5**, the utility of the FoldFx model is illustrated with a hypothetical biological network of interacting proteins of a proteome. A biological network is defined by nodes or proteins (indicated by spheres) and edges or protein interactions within the network (indicated by the lines between the spheres). A set of hypothetical nodes is positioned in **Figure 5** according to the corresponding proteins' folding energetics: their stabilities, folding rates, and misfolding rates at a given proteostasis network capacity. The energetic parameters for the nodes in **Figure 5** were randomly generated with the constraint that they be physically plausible (e.g., it was assumed that folding could not take place on a timescale faster than 1 μs). The proteostasis boundary was determined by arbitrarily setting the concentrations of the proteostasis network components to reasonable values (46) and solving the FoldFx model (mathematically analogous to the FoldEx model). This solution was then used to determine which region of the space, defined by the protein stability, folding rate, and misfolding rate, yielded a more than minimal fraction of functional protein (this fraction was set to 0.1 for **Figure 5**). The proteostasis boundary is the surface that surrounds this region.

In a healthy cell, nodes are positioned within the limits defined by the proteostasis boundary

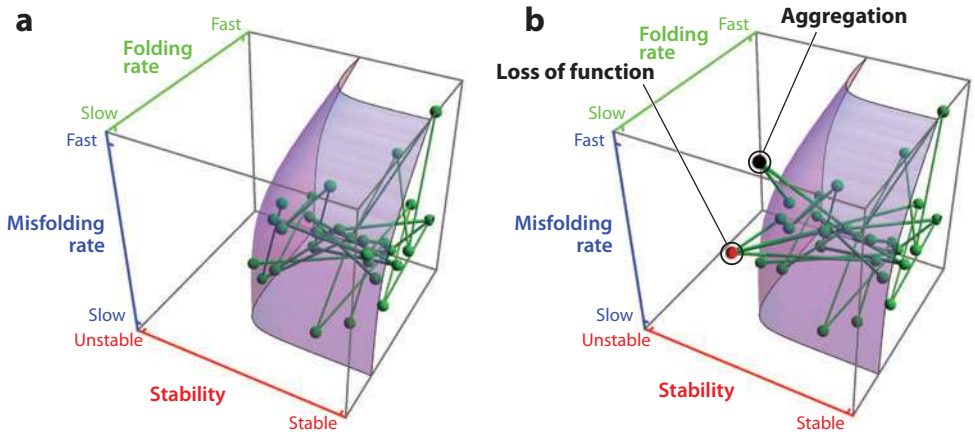

Figure 5

A hypothetical network of interacting proteins as viewed in the FoldFx model. Each node (or *sphere*) in the network represents a corresponding protein's folding energetics, and each connection (*line*) represents a physical or functional interaction. The surface represents a proteostasis boundary, which, for the sake of simplicity, is shown as being the same for all of the proteins in the network. (*a*) All of the nodes are within the proteostasis boundary in a healthy cell. (*b*) Mutations or aberrant posttranslational modifications can alter the folding energetics of stable proteins, making their corresponding nodes fall outside the proteostasis boundary. This can lead to either loss of function (*red node*) or aggregation (*black node*).

(**Figure 5*a***, green nodes). Some nodes fall well within the proteostasis boundary, indicating that they are fast folding and thermodynamically stable proteins. These proteins may not require significant biological assistance and will likely tolerate major changes to the capacity of the proteostasis network. In contrast, less stable, slowly folding, and/or rapidly misfolding proteins are closer to the proteostasis boundary, and their ability to achieve biological function is thus likely to be much more sensitive to proteostasis network capacity.

When the folding energetics of a given protein are adversely affected by mutation or by oxidative stress (e.g., a lipid aldehyde modification), it is reasonable to hypothesize that the protein of interest would move outside the limits defined by proteostasis boundary. In this case, the protein can be prone to either degradation (**Figure 5*b***, red node) or aggregation (**Figure 5*b***, black node). Of particular interest are those proteins lying close to or at the proteostasis boundary. We propose that these proteins will be particularly sensitive to modest changes in the capacity and composition of the components of the proteostasis network that influence the shape of the proteostasis boundary.

In the FoldFx view, the position and shape of the proteostasis boundary suggests that many sequences that are competent to fold in vitro (in buffer at low protein concentration) may not be useful for function in vivo, as has been observed for many mutant proteins and protein fragments, especially if a relatively high concentration of protein is required for function as it would exacerbate aggregation (45). Although the actual distribution of nodes defining a biological network relative to the proteostasis boundary is influenced by both the folding energetics and the biology of the network, we suggest that proteins that are marginally stable and lie on or close to the proteostasis boundary may participate in the reversible assembly of dynamic protein complexes (11, 47). Moreover, given the high variability of the proteostasis network capacity in different cell types (**Figure 3**), housekeeping enzymes that are critical for all cells may be largely insensitive to the shape of the proteostasis boundary because they are energetically stable and fall deep within its embrace (48).

The apparent plasticity of the proteostasis boundary illustrates how critical the proteostasis network is to protein folding efficiency and also to the life of a cell. This hypothesis is consistent with recent evidence that suggests the proteostasis network does not possess significant excess capacity; rather, it is finely tuned and offers just enough capacity for the protein folding load (17, 49, 50). Although at first glance confounding, this is consistent with the key role of the proteostasis network in defining functionality in diverse cellular environments. Thus, by setting the proteostasis boundary as a threshold for generating folded and functional proteins, the proteostasis network can create and maintain functional proteins in response to the local environment.

Of course, the chaperone and folding enzyme components that contribute to the location and shape of the proteostasis boundary are themselves part of the biological network. We expect that they would generally lie well within the proteostasis boundary and would act as hubs (highly connected nodes in the biological network) that have numerous, but weak, interactions with multiple proteins that can be altered in response to stress. As such, they have been suggested to promote stability of the biological network, buffer noise, and regulate signaling pathways that would otherwise spuriously modify the network (see Reference 51 for further details).

FOLDING DISEASES AND THE PROTEOSTASIS BOUNDARY

Unlike in vitro assessment(s) of folding and function, biology needs to distinguish pathological disorder from functional disorder encoded in the polypeptide sequence. It is not yet understood how this is accomplished. The FoldFx model and the proposed concept of a proteostasis boundary provide a framework to assess the impact of folding defects on proteostasis. These ideas allow us to better understand new pharmacologic approaches for maintaining health and countering disease by either adjusting the energetics of the protein fold, the capacity of the proteostasis network, or both.

The first question we need to ask is: When is a protein misfolded from a functional perspective? Here, we must consider the stability of the fold and proteostasis network capacity in light of the shape and position of the proteostasis boundary that will be required for activity. Thus, we would suggest that a key concern in human health is how the local environment, a mutation, or a posttranslational modification alters a protein's ability to function in a biological network defined by the proteostasis boundary.

In inherited diseases associated with a folding deficiency, it is well established that a change in the amino acid sequence of a protein can significantly alter folding energetics and, therefore, the position of the protein relative to the proteostasis boundary. For conservative missense mutations, the substitution may be neutral. In other words, it has little influence on folding kinetics or thermodynamics. In this case, it would have little effect on function unless it eliminates a catalysis-critical or binding-critical residue. A less conservative missense mutation or an amino acid deletion often alters the kinetics or the thermodynamics of folding. Such a mutation could move a protein outside the proteostasis boundary, where it may become susceptible to substantial misfolding, aggregation, and/or degradation. The effect of the protein being excessively degraded could lead simply to loss of that protein's function, in which case only one node in the network would be affected (**Figure 6*a***, red node). The effect could also be more far reaching if, for example, the protein was involved in interactions that stabilized other proteins. In the latter case, some or all of the other proteins with which the mutant protein interacts may now fall outside the proteostasis boundary in response to destabilization (**Figure 6*b***, multiple red nodes), further compromising cell or organism function.

Alterations in the composition or concentration of proteostasis network components in disease could have a global effect on the folding of the proteome, depending on how

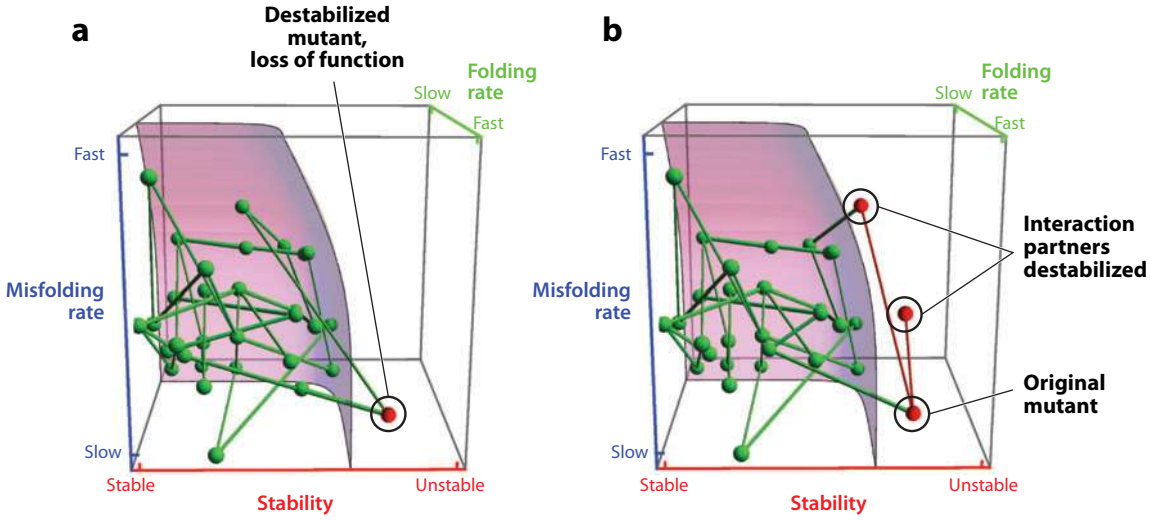

Figure 6

Plots illustrating the effect of a destabilizing mutation on protein function. (*a*) The destabilized protein falls outside the proteostasis boundary and is degraded, but the loss of function is limited to the destabilized protein itself. (*b*) As in panel *a*, degradation of the original mutant destabilizes the proteins it interacts with, leading to further loss of function and multiple nodes being repositioned outside the proteostasis boundary.

many proteins are close to the proteostasis boundary in a given cell type. We predict that a decline in the capacity of a core proteostasis network component, such as Hsp70, Hsp90, or proteasome subunits, could have far-reaching and toxic effects. In contrast, we posit the sphere of influence of disrupting more specific components (e.g., Hsp90 cochaperones, such as immunophilin isoforms) will be restricted to those proteins whose folding pathways depend specifically on the activity of those components. This interpretation is consistent with the considerable expansion of cochaperone complexity with increasing eukaryotic cell complexity. If folding capacity, as defined by the proteostasis boundary, is increased by overexpressing proteostasis network core or accessory chaperone/cochaperone components (as occurs normally but transiently, for example, in the UPR and HSR), the new proteostasis network is likely to be protective, as is observed in stress responses (34–36). We propose that manipulating degradative activities (e.g., ubiquitin-mediated proteasomal, lysosomal, and autophagy pathways) is potentially a double-edged sword. Degradation is a normal activity that is used to control a variety of activities, including cell cycle and developmental programs, or removal of misfolded or aggregated toxic proteins. Thus, decreasing degradation could, in principle, increase the concentration of folded, functional protein; alternatively, it could also increase the concentration of misfolded, toxic species. Conversely, increasing the activity of degradation pathways could promote removal of toxic, pathology-associated misfolded and aggregated protein, but it could also lead to global destabilization of the biological network by overzealous removal of one or more proteins, which are close to the proteostasis boundary owing to their sequences but are critical for normal function and survival.

Given the FoldFx view, we now need to consider the possibility that the clinical features of many inherited and sporadic diseases, as well as responses to stress encountered during aging, are consequences of changes in the proteostasis network (1). We suggest that such changes in the proteostasis network with aging and the corresponding changes in the shape of the proteostasis boundary, although variable for different compartments and cell types, alter

the ability of a cell to handle its proteostasis load. Thus, we propose that pharmacologic up- or downregulation of the capacity of the proteostasis network provides an avenue for intervention in diseases of proteostasis, as discussed below, where we summarize the increasing evidence that supports this hypothesis.

THE PROTEOSTASIS BOUNDARY AND PHARMACOLOGIC CHAPERONES

The primary challenge in ameliorating loss-of-function misfolding diseases is to identify small-molecule pharmacologic agents that selectively or specifically enhance protein stability and function. One could envision moving a node from outside the proteostasis boundary to inside by stabilizing the borderline protein or by expanding the proteostasis network capacity and thus expanding the proteostasis boundary to envelop the destabilized protein, i.e., the problematic node. Herein, we focus on two classes of small molecules that are becoming increasingly recognized in the literature and that can accomplish these goals: pharmacologic chaperones (PCs), which move the node by stabilizing the destabilized protein of interest, and proteostasis regulators (PRs), which expand the proteostasis boundary to surround the borderline protein by enhancing the capacity of the proteostasis network.

Small-molecule PCs function by binding to the destabilized target protein and thus stabilizing it. PCs, in general, should be efficacious within the context of most, but not all, existing proteostasis networks depending on how destabilized the metastable protein is. For many misfolding-prone proteins, PCs should be able to move the protein within the proteostasis boundary, thereby increasing function by elevating the concentration of folded functional protein. In principle, there are three ways to correct a misfolding disease with PCs: The misfolding-prone protein can be thermodynamically stabilized (**Figure 7*a***, point is moved along the stability axis); or the folding rate can be increased by a PC that stabilizes the folding transition state (**Figure 7*a***, point is moved along the folding rate axis); or the misfolding rate can be decreased by stabilizing the native state (**Figure 7*a***, point is moved along the

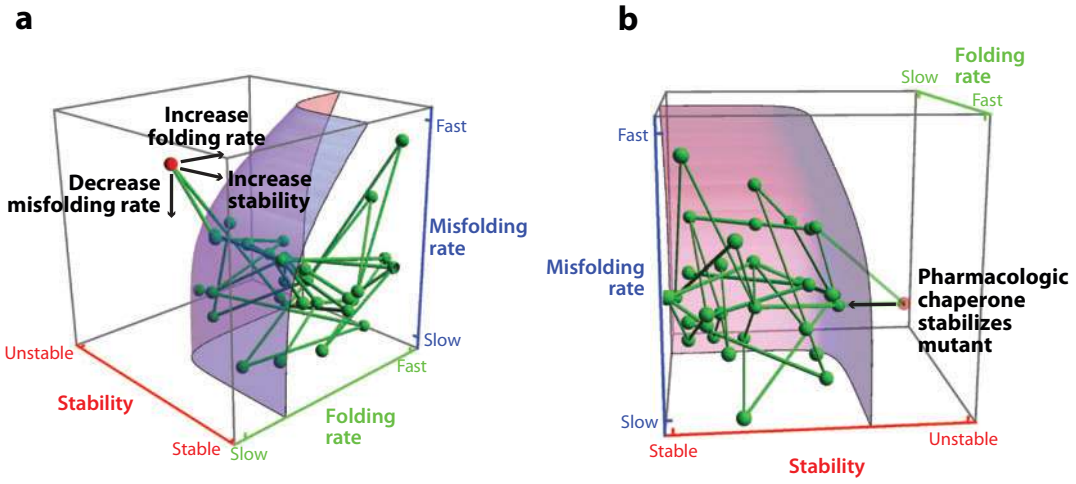

Figure 7

The effect of pharmacologic chaperones on protein folding in the FoldFx model. (*a*) Pharmacologic chaperones bind to a protein, directly affecting its folding energetics. This binding can help rescue a protein with defective folding energetics by increasing its folding rate, decreasing its misfolding rate, increasing its stability, or any combination thereof. (*b*) Increasing protein stability, illustrated in this rotated plot, is the mechanism of action of most known pharmacologic chaperones.

misfolding rate axis). To date, only the first and third mechanisms have been demonstrated experimentally, although the potential remains for other classes of PCs to be developed.

Known classes of mutant proteins that can be corrected by PCs include G protein–coupled receptors (52), neurotransmitter receptors (53), glycosidases, and analogous enzymes associated with lysosomal storage diseases (LSDs) (54–56), and, potentially, the cystic fibrosis transmembrane conductance regulator (CFTR) (57, 58). In cystic fibrosis, the ΔF508 deletion in CFTR disrupts its folding and targets the protein for degradation in the ER. Putative PCs that act on the CFTR include polyaromatic compounds (59) that have been shown to stabilize the transmembrane helices (60) in order to stabilize CFTR for export from the ER. This activity would be visualized in terms of the FoldFx model as shown in **Figure 7*b***, in which a red node (representing a loss-of-function mutant protein) that lies outside the proteostasis boundary is moved inside the barrier by thermodynamic stabilization of the ΔF508 CFTR fold. A second class of PCs, referred to as potentiators, cannot stabilize the fold sufficiently for export from the ER but, once CFTR is at the cell surface, will promote stabilization of the channel and thereby increase conductance, perhaps through decreased degradation and/or allosteric mechanisms favoring interaction with regulatory kinases and phosphatases (61, 62). The Vertex 770 potentiator has just completed highly successful phase II clinical trials where a marked recovery in sweat chloride, nasal potential, and lung capacity was observed for the G551E CFTR variant that is exported from the ER normally but is inactive at the cell surface (**http://www.cff.org/**). It has been suggested that a combination of a corrector PC, to promote folding and export, and a potentiator PC, to enhance activity of the partially destabilized channel, could be the key to effectively treating cystic fibrosis patients (63). Similarly, numerous antagonists and agonists for ion channels and neurotransmitter receptors that affect their function may do so partly by functioning as PCs to favor altered folding and trafficking.

The use of PCs is on firm mechanistic ground and is particularly promising for the treatment of LSDs, including Fabry's, Pombe's, Tay-Sachs, and Gaucher's diseases (54, 55, 64). Several PCs that bind to and demonstrably stabilize the mutant lysosomal enyzmes susceptible to excessive degradation are in various phases of clinical trials for different LSDs. LSDs are largely loss-of-function diseases caused by the excessive ER-associated degradation of destabilized variant lysosomal enzymes. The resulting loss of function leads to substrate accumulation in the lysosome and, hence, pathology. Like the ΔF508 CFTR variant, most misfolding-prone LSD-related variant proteins do not trigger the UPR because the proteins are removed by ER-associated degradation, leaving the proteostasis network unperturbed. However, it is now appreciated that numerous ligands, many of which are inhibitors, that bind to the active sites of specific destabilized lysosomal enzymes stabilize their folds in the ER (**Figure 7*b***). Stabilization of β-glucocerebrosidase variants leads to enhanced folding and trafficking of the protein to the lysosome where it is more stable and functional (albeit with reduced specific activity) owing to the lower pH of this organelle relative to the neutral pH/environment of the ER and the high concentration of substrate (55, 65, 66). As mentioned above, it is expected and observed that not all β-glucocerebrosidase variants respond to PC therapy. Highly destabilized mutants of the enzyme are degraded so efficiently that the concentration of folded enzyme available for PC binding is simply too low for a PC to have an observable effect on the folding equilibrium constants. Destabilized glucocerebrosidase mutants require a different approach to improve their export from the ER and downstream function, which involves increasing proteostasis network capacity (see below).

A special class of PCs has proven to be useful for stabilizing proteins against misfolding and amyloid fibril formation in degenerative diseases by differentially stabilizing the non-amyloidogenic native state over the misfolding transition state. These so-called

kinetic stabilizers function by significantly decreasing the misfolding rate. Two chemical classes of kinetic stabilizers are currently being used in human clinical trials to ameliorate the transthyretin (TTR) amyloidoses (66, 67; **http://www.clinicaltrials.gov**). TTR is synthesized as a monomer and tetramerizes before ER export. Mutations that destabilize the tetramer result in its disassembly to monomers in the serum. These monomers can partially unfold and undergo amyloid formation at a rate that increases with increasing TTR monomer concentration. Kinetic stabilizers (66, 67) bind to TTR tetramers, preventing dissociation under physiological conditions, thereby decreasing the monomer pool in the serum, substantially slowing and, in some scenarios, preventing protein aggregation. Thus, TTR kinetic stabilizers show how gain-of-toxic function can be avoided by a special class of PCs that kinetically stabilize the native non-amyloidogenic state of proteins.

MOVING THE PROTEOSTASIS BOUNDARY WITH PROTEOSTASIS REGULATORS

Proteostasis regulators (PRs) include small molecules that offer the advantage that one compound can be used to expand the proteostasis boundary for numerous misfolding-prone proteins that use a common set of proteostasis network components associated with a particular cell type, compartment, or activity (1). PRs can favor folding by adjusting the composition, concentration, and, thus, the capacity of the proteostasis network by expanding the proteostasis boundary (**Figure 8**). We speculate that PRs could also be discovered that collapse the proteostasis boundary by upregulating components of the proteostasis network that favor unfolding and degradation (**Figure 8**), although we are unaware of any such compounds.

In general, we hypothesize that the proteostasis boundary could be expanded by changing the levels and/or activities of core (Hsp70/Hsp90) proteostasis network pathways. Expansion can occur, for example, by inducing cellular Ca^{2+} distribution changes (68) or by inducing the UPR or HSR signaling pathways (34–36), all of which are known to increase proteostasis network capacity (see below). A selective expansion of the proteostasis boundary could also be achieved by PRs that directly target one or a few of the diverse Hsp40 isoforms that modulate the folding of specific misfolding-prone proteins. Instead of inducing the UPR or HSR, the Hsp70 or Hsp 90 core systems could be more selectively altered by targeting one or a few of the many cochaperones that differentially regulate interactions with desired misfolding-prone proteins. We propose that in this way PRs could function as rheostats to fine-tune the proteostasis boundary in a particular compartment in the cell with respect to a subset of proteins.

PRs could also potentially be used to precondition the proteostasis network to more effectively handle metabolic stress (69) and aging by increasing the protective capacity of the proteostasis network prior to an insult. Generally speaking, PRs could be used to upregulate a pathway within the proteostasis network to increase proteostasis capacity, just as the cell normally uses transient proteostasis signaling pathways such as the UPR and HSR to respond to stress and reestablish proteostasis. Below we briefly highlight the potential utility of a few of the compounds in the growing PR toolbox.

The drug salubrinal (70) uses aspects of one arm of the UPR to protect cells against protein misfolding stress. Phosphorylation of eukaryotic initiation factor 2 (eIF2) is accomplished by at least four kinases, including the UPR kinase PERK in response to unfolded protein accumulation in the ER (35, 36). Phosphorylated eIF2 protects cells by blocking translation, thereby reducing the folding load. Salubrinal activates the PERK-ATF4 arm of the UPR downstream of PERK by inhibiting dephosphorylation of eIF2, thereby easing the folding load and avoiding the apoptosis that is typically associated with sustained activation of the PERK-AFT4 arm of the UPR (70, 71).

PRs that activate the HSR include triptolide, quercetin, and celastrol, all heat shock

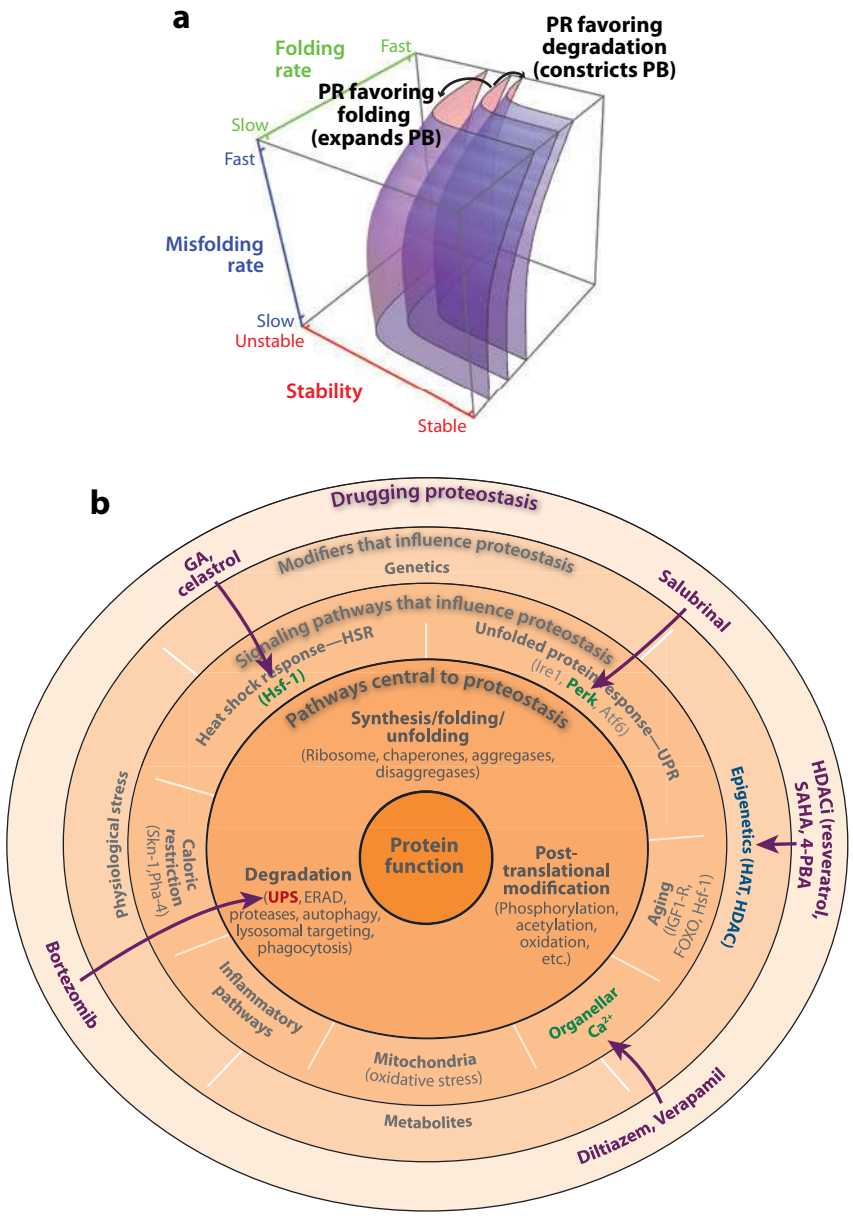

Figure 8

The effect of proteostasis regulators (PRs) on the proteostasis boundary (PB). (*a*) PRs can either expand the proteostasis boundary, favoring protein folding, or constrict the proteostasis boundary, favoring degradation. The expanded and contracted proteostasis boundaries resulted from increasing or decreasing, respectively, the concentrations of chaperones or degradation machinery. (*b*) Influence of select PRs (*outer layer*, in *purple font*) on managing proteostasis. The listed drugs and their effects on the indicated pathways and components described in **Figure 2** are highlighted in the text.

transcription factor 1 (HSF1) enhancers (72–74). Celastrol was originally identified in a screen for compounds that were neuroprotective in Huntington's disease models (75). Evidence now suggests that HSF1 production is increased by reduced insulin growth factor-1 receptor (IGF1-R) signaling, which protects against neurodegeneration and improves longevity (see below) (1). Although celastrol's mechanism of action is not fully elucidated, recent studies suggest it to be a thiol-reactive molecule that triggers stress responses through this chemical activity (72). Reflecting this chemical activity, celastrol has been found to inhibit the activity of the HSF1 repressor Hsp90 (76), to inhibit NFκB (77), and to inhibit the proteasome (78) as well as to modulate Ca^{2+}-signaling pathways (72). Celastrol also appears to be useful as an adjuvant for arthritis (79) and for preventing cancer through Hsp90-dependent steps (see below). The different responses of various cell types to celastrol could be a consequence of the different demands that cells place on their unique proteostasis networks and the composition of the signaling pathways that control proteostasis. A similar possibility could apply to nonsteroidal antiinflammatory drugs (NSAIDs), including aspirin and acetaminophen, which inhibit the cyclooxygenases COX 1 and 2. NSAIDs have antioxidant properties (80) and have been suggested to modulate Hsp70 and Hsp90 chaperones, as well as HSF1 signaling pathways (81). Given the emerging complexity of their targets and the broad range of indications exhibited by these compounds, we speculate that NSAIDs, like HSF1 pathway modulators, may have unexpected influences on the position and/or shape of the proteostasis boundary.

Ca^{2+}-sensitive signaling pathways control cell function at multiple levels (38, 39). Recent evidence suggests that alterations of intracellular Ca^{2+} levels can regulate proteostasis network capacity in the ER and, possibly, in other cellular compartments and hence alter the position and shape of the proteostasis boundary (68). Inhibition of L-type Ca^{2+} channels in the plasma membrane using either diltiazem or verapamil partially restored β-glucocerebrosidase folding, trafficking and function in Gaucher's disease patient-derived fibroblasts (68). Structure-activity relationship studies of analogs showed a clear correlation between Ca^{2+} antagonism and PR function. This observation led to the suggestion that the beneficial effects of diltiazem and verapamil are mediated by increased ER Ca^{2+} concentrations, likely influencing the activity of Ca^{2+}-dependent ER chaperones like calnexin and calreticulin, and, indirectly, the function of BiP, the major Hsp70-related chaperone, through Ca^{2+}-linked mechanisms. Because diltiazem and verapimil increase the ER proteostasis network capacity and expand the proteostasis boundary, these PRs have the ability to enhance the folding, trafficking, and function of non-homologous mutant lysosomal enzymes associated with other distinct LSDs, emphasizing the feasibility of discovering one PR that would be useful for the treatment of multiple diseases (68). Moreover, recent studies have established a link between Ca^{2+} signaling and redox activity within the ER, suggesting that protection from oxidative stress may also be a factor (82).

PRs that are known to activate the UPR are also useful in rescuing the folding, trafficking, and function of unrelated, misfolding-prone lysosomal enzymes (83). Co-application of MG-132 (a PR that functions as a proteasome inhibitor and as a UPR activator) or celastrol (a PR that is both a UPR and a HSR activator) with RNAi to separately inhibit each of the three arms of the UPR and the HSR revealed a strong dependence of the action of these PRs on two or more arms of the UPR, but not the HSR. Enhancement of enzyme folding, trafficking, and activity by these PRs was further increased by β-glucocerebrosidase-targeting PCs, even for a variant that cannot normally be rescued by PCs (83). In fact, the activities of PCs and PRs exhibited synergy in several lysosomal storage disease contexts. Notably, such results suggest that these PRs improve the proteostasis network capacity, enabling production of more folded β-glucocerebrosidase for PC binding, thereby significantly increasing folded enzyme

concentration and subsequent export to the lysosome (83). It is likely that this synergistic rescue of proteostasis arises from expanding the proteostasis boundary with the PR, while simultaneously stabilizing the increased population of the folded state by PC binding to the native state of the folding-compromised enzyme, moving it even further within the proteostasis boundary. We anticipate that PRs and PCs, which offer mechanistically distinct solutions to enhancing proteostasis capacity, can be combined to address challenges to the proteostasis network that could not be met as well by either type of agent alone.

An emerging category of PRs that presumably function through regulation of transcription are the HDAC inhibitors (HDACi) that modulate the epigenome (84). HDACi appear to modulate transcription, and thereby proteostasis, by preventing histone deacetylation (85–88). HDACs comprise a group of 18 enzymes. They are divided into three major classes, the Zn^{2+}-dependent classes I and II, and the NAD^{+}-dependent class III enzymes (sirtuins) (89–93), the latter being sensitive to the natural product, resveratrol (94, 95). HDACs function posttranslationally to regulate the level of acetylation and hence the activity of transcription factors and other proteins, such as the chaperone Hsp90 (85, 96, 97) and HSF1, the latter illustrating the integral role of HDACs in proteostasis (98). Increasing evidence suggests that HDACis, such as tubacin, appear to strongly influence the proteostasis network. Tubacin targets HDAC6. HDAC6, through its ability to regulate the acetylation status and activity of Hsp90, has been proposed to control the cellular response to stress through the interaction of Hsp90 with HSF1, which in turn controls the cellular response to multiple cytosolic stress events. Similarly, 4-phenylbutyrate (4-PBA), a putative low-affinity HDACi, has been shown in mouse models to provide benefit for numerous misfolding diseases that challenge the proteostasis network, including metabolic syndrome (99), cystic fibrosis (100, 101) and α-1-antitrypsin (α1AT) deficiency (102), possibly by expanding the proteostasis boundary (**Figure 8**). Changes in the proteostasis boundary in response to HDACi may also protect organisms from aging and aging-associated neurodegenerative diseases (see below).

Autophagy, a proteostasis pathway enabling protein degradation, plays an important role in cell maintenance by removing intracellular aggregates and misfolded proteins by delivering them to lysosomes. The autophagic pathway has three branches to facilitate this process, referred to as macroautophagy, microautophagy, and chaperone-mediated autophagy (103, 104). Macroautophagy is negatively regulated by the mTOR pathway, which is inhibited by rapamycin (105–107). Upregulation of mTOR is protective against neurodegenerative disease (see below) (108, 109). Thus, we propose that PRs, such as rapamycin, that regulate the proteostasis boundary via their effects on autophagy pathways to remove unfolded, misfolded, or aggregated protein could be useful for a broad range of diseases involving the accumulation of aggregation-prone proteins in the cytosol and trafficking compartments. The latter includes Z-variant α1AT aggregates, which accumulate in the ER and are eliminated by macroautophagy (102, 110, 111). Recent studies reveal that Ca^{2+}-sensitive signaling represents another pathway by which PRs could control autophagy (112).

Although we have focused on PCs and PRs that modify the proteostasis network in a cell-autonomous fashion, organismal biology operates in the context of interconnected tissue and organ systems. Thus, the responses of proteostasis networks need to be globally coordinated. Recent work suggests that organismal control of the proteostasis network can be cell nonautonomous and centrally regulated by neuronal signaling pathways. Exposure of *Caenorhabditis elegans* to heat stress revealed that the response of somatic cells required only one thermosensory neuron, AFD (42, 113). Thus, we speculate that coordinated regulation of the proteostasis boundary within and between organ systems serves to integrate behavioral, metabolic, and stress-related

pathways. PRs that mimic the activity of these cell-nonautonomous pathways remain to be developed, although incretin analogs may have PR properties (see below).

THE PROTEOSTASIS NETWORK IN AGING

Processes associated with aging have direct signaling-mediated connections to the proteostasis network and are, therefore, capable of moving the proteostasis boundary, potentially explaining why so many loss- and gain-of-function diseases are triggered or exacerbated by aging (**Figure 8**). Substantial progress has been made in understanding factors contributing to aging through the IGF1-R signaling pathways, dietary restriction, mitochondrial respiration, (114–117) and, more recently, the Elt3, -5, and -6 signaling pathways, the latter having genetic links to IGF1-R pathways (118). Longevity is markedly extended by reducing the level or activity of IGF1-R signaling in worm, fly, and mouse models (114–117). IGF1-R is a negative regulator of both FOXO and HSF1 signaling pathways that directly influence the proteostasis network. One of several current models for the effects of aging on organisms is that the proteostasis network becomes burdened by the accumulation of proteins modified by reactive oxygen species or oxidative metabolites, particularly in nondividing cells such as the neuron. Such modified proteins have a tendency to misfold and/or aggregate, placing a significant demand on the proteostasis network. To meet this challenge, it has recently been demonstrated that reduction of IGF1-R signaling results in HSF1 activation and upregulation of protective features of the proteostasis network. Thus, reduction in IGF1-R signaling appears to compensate for what would otherwise be a gradual, aging-related collapse of proteostasis owing to an increased misfolding/aggregation load. Intriguingly, a reduction in IGF1-R signaling could even "precondition" aging cells for later proteostasis challenges (1). Thus, we suggest that modulation of the proteostasis network by PRs that reduce IGF1-R signaling or upregulate HSF1 or FOXO are likely to be effective for aging-associated degenerative diseases linked to protein aggregation by maintaining or expanding the proteostasis boundary (**Figure 8**) and should, therefore, be of high interest to biotechnology and pharmaceutical industries.

OBESITY AND THE PROTEOSTASIS NETWORK

Obesity is a major problem in industrialized societies and negatively influences the response of the normally adaptable proteostasis network to stress, including that associated with metabolic disease (99, 119–121). A high-fat diet, a lack of exercise, and genetic influences strongly impact the ability of the proteostasis network to maintain the function of β-cells in response to the increased demand for insulin and in response to dysregulation of insulin signaling (36, 122, 123). A large body of evidence now suggests that when the rate of insulin production in the ER exceeds the cell's capacity to fold and secrete it, numerous signaling pathways, including the UPR, attempt to prevent toxicity and cell death. Thus, not only does insulin folding fail, but we speculate that the proteostasis boundary eventually collapses in response to the unmanageable misfolding load. Thus, the β-cell consequently loses its ability to generate and maintain the structure and function of other proteins essential for survival. This is illustrated in terms of the FoldFx model in **Figure 9**. The collapse of proteostasis capacity in the β-cell is exemplified not only by deposition of aggregates of the peptide hormone, amylin (124, 125), but also by β-cell death, presumably resulting from a sustained activation of the PERK-ATF4 arm of the UPR, which is linked to death pathways through the transcription factor CHOP (34–36, 126). Although the most common diabetes drugs provide temporary relief, they do so by further increasing insulin production in β-cells and, therefore, could exacerbate the problem in the long term. In contrast, HDACi, including 4-PBA (99, 127), and activators of sirtuins, such as resveratrol

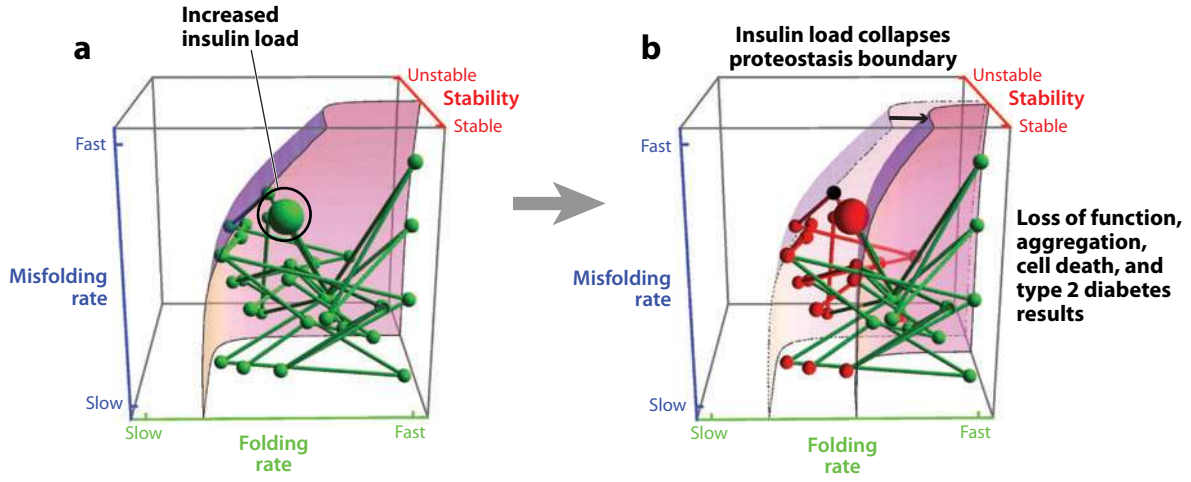

Figure 9

A potential mechanism for the pathogenesis of type 2 diabetes visualized in terms of the FoldFx model using a hypothetical protein interaction network. (*a*) Increased synthesis of insulin (represented as a *large node*) challenges the proteostasis network. The insulin load saturates the proteostasis network, leaving little capacity for maintaining the normal protein folding load. (*b*) The proteostasis boundary constricts, leading to loss of β-cell function, protein aggregation (of the peptide hormone amylin in particular, *black node*), β-cell death, and disease. Because amylin is an intrinsically disordered peptide, the assignment of values for its folding kinetics and thermodynamics reflects its dynamic equilibrium between degradation-competent and -incompetent states.

and its analogs (94, 128–130), restore β-cell homeostasis in mouse models of type 2 diabetes, perhaps by modulation of the highly compromised proteostasis network. Intriguingly, mimics of the secreted incretin class of hormones, including the glucagon-like peptide-1 (GLP-1) receptor agonist exenatide (131) or inhibitors of dipeptidyl peptidase-IV that block GLP-1 degradation (132), may function by rebalancing the proteostasis network in the β-cell. Moreover, recent studies revealed that cell-nonautonomous inter-organ communication between the liver and the pancreas via neuronal pathways controls β-cell function and insulin secretion (133). Thus, consistent with the response of worms to heat stress through neuronal signaling pathways (42), compounds such as exenatide and other polypeptide hormones may fall into the broad category of cell-nonautonomous PRs and present a more global biological solution for augmentation of the proteostasis network to preserve insulin synthesis, protect β-cells from apoptosis, and promote β-cell proliferation.

CANCER AND THE PROTEOSTASIS NETWORK

The unregulated cell division exhibited by cancer cells requires exceptionally high rates of protein synthesis and maintenance that are out of balance relative to the normal, fully differentiated state, taxing the proteostasis network (134–136). Hsp90 is a highly abundant chaperone that has >300 clients, including many kinases involved in cell proliferation (**http://www.picard.ch/downloads/downloads.htm**). Unlike Hsp70, which is involved in cotranslational protein folding, Hsp90 is largely involved in late events in protein folding pathways, where it maintains proteins in near-native states for functional purposes. Hsp90 is sensitive to a number of inhibitors that belong to the ansamycin class of antibiotics including geldanamycin and its derivatives, radicicol, and a series of derivatives based on purine and pyrazole scaffolds (137–139). Interestingly, cancer cells are highly sensitive to PRs inhibiting Hsp90. Hsp90 inhibitors are currently the focus of multiple anticancer

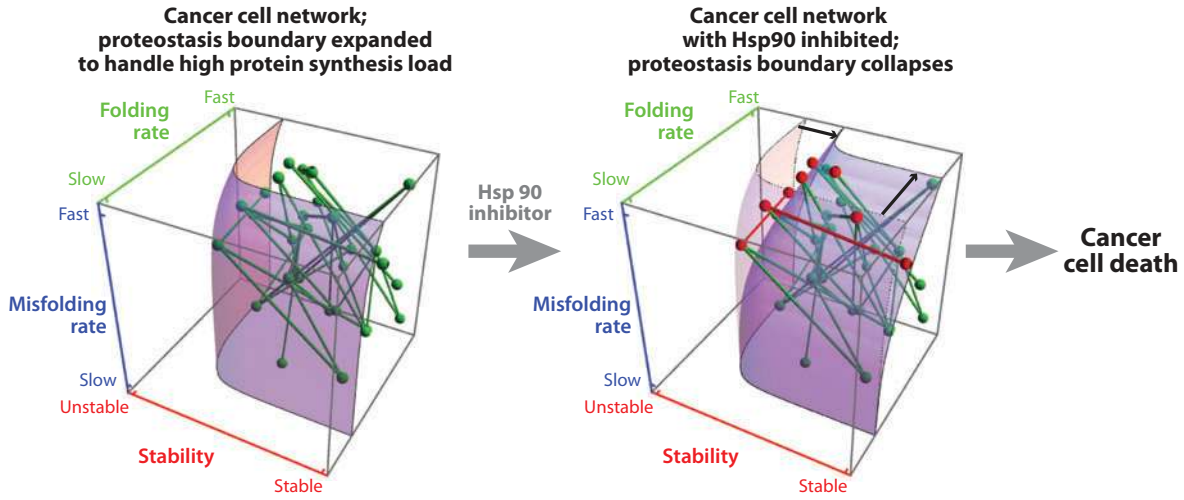

Figure 10

Treatment of cancer using Hsp90 inhibitors visualized in terms of the FoldFx model.

clinical trials (136, 140). We suggest that disrupting Hsp90 function in a cancer cell would likely contract the proteostasis boundary in the FoldFx model (**Figure 10**). This would lead to loss of function for many proteins with marginal protein folding energetics, thereby killing the cancer cell, possibly without significantly impacting normal cell physiology.

Compounds that modulate oxidative folding and metabolic pathways, the latter being a central feature in proteome maintenance during stress and aging, are also likely candidates as PRs to treat cancer. Arsenicals found in traditional Chinese medicine and used for treatment of cancer (141) function as PRs presumably by compromising the redox potential of the cytosolic folding environment. As a consequence, we predict that they significantly challenge the capacity of the proteostasis network to fold proteins with disufide bonds and, hence, the ability of the cancer cell to maintain viability (142). Although modulation of oxidative stress remains a major challenge for PR development, compounds influencing S-nitrosylation, and/or inflammatory signaling pathways, including NF-κB or PERK signaling via activation of ATF4 and Nrf2 transcription factors (41), and Ca^{2+}-signaling pathways that are linked to redox pathways may represent major opportunities to reduce or prevent disease (143).

PRs that inhibit degradation are now part of an established clinical approach to prevent growth of specific classes of cancer. Here, PRs that are proteasome inhibitors illustrate the value of manipulating the proteostasis boundary to promote cell death (30, 144, 145). Proteasome-inhibiting PRs decrease the degradation rate of misfolded proteins. The resulting increase in the concentration of misfolded proteins likely saturates the proteostasis network, collapsing the proteostasis boundary below a threshold that can sustain cell viability (see **Figure 10**). For example, in multiple myeloma, the proteasome inhibitor bortezomib selectively kills the cancerous plasma cells that produce large amounts of immunoglobulin, sometimes resulting in remission of the cancer (146, 147). In terms of the FoldFx model, we speculate that this selectivity arises because myeloma cells are already challenged with a high protein folding load, and proteasome inhibition further diminishes the capacity of the proteostasis network to maintain the proteostasis boundary at a functional level. The effect could be similar to that of treating other types of cancer cells

with Hsp90 inhibitors, although different components of the proteostasis network are targeted (**Figure 10**).

AGE-RELATED DEGENERATIVE DISEASES AND PROTEOSTASIS

Although we know little about maintenance of the intrinsically disordered proteome, it is clearly important as intrinsically disordered proteins form the aggregates that trigger or exacerbate the clinically most important neurodegenerative diseases, including the aggregation of Aβ associated with Alzheimer's disease, α-synuclein associated with Parkinson's disease, and the exon1 fragment of huntingtin associated with Huntington's disease. Longevity signaling pathways that influence cytosolic proteostasis have been shown to have strong effects on the progression of gain-of-toxic-function neurodegenerative diseases in animal models (1).

In a *C. elegans* model of Aβ proteotoxicity, inhibiting the IGF1-R signaling pathway, which negatively regulates HSF1, revealed two ways in which a cell can use the proteostasis network to protect itself (148). The first activity, enabled by upregulation of HSF1 transcription factor activity, is a disaggregation activity that appears to reduce aggregate load. A second pathway, also downstream of IGF1-R signaling, utilizes the FOXO transcription program, apparently to enhance the formation of large aggregates, thereby protecting the cell from smaller, more toxic oligomers by placing these out of reach of the functional environment defined by the proteostasis boundary (148, 149). The putative effects of these pathways on proteostasis are illustrated in terms of the FoldFx model in **Figure 11**. It will be interesting to discern whether the proteostasis network generally uses aggregation-promoting activities to mitigate the effect of acute and/or chronic failures of proteostasis when the capacity of the protective disaggregation/degradative pathways becomes ineffective or saturated (150).

A second example of the proteostasis network protecting the cell from aggregation-associated proteotoxicity is observed in *C. elegans* models harboring huntingtin polyQ expansions (17, 50, 151, 152). Here, the cytotoxicity triggered by a polyQ aggregate load is exacerbated by decreased HSF1 activity and ameliorated by reduced IGF1-R signaling that increases HSF1-mediated transcription. Moreover, introduction of another misfolding-prone protein into *C. elegans* harboring huntingtin

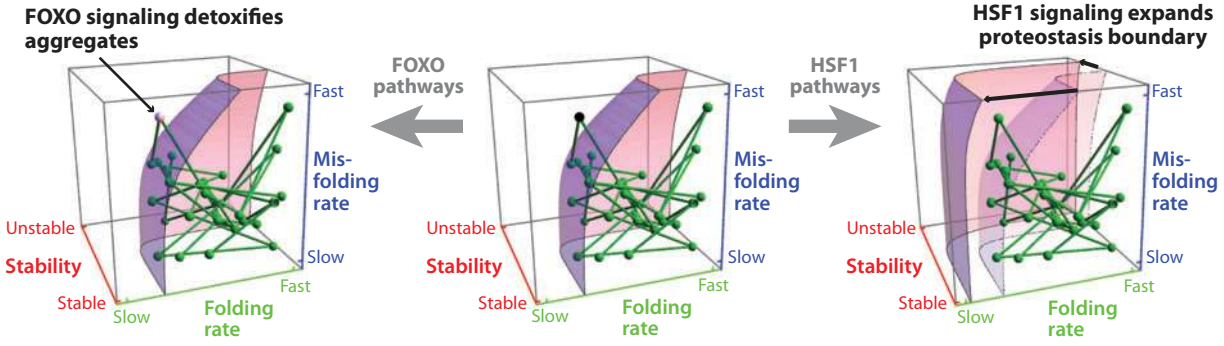

Figure 11

The effect of activating HSF1 pathways on protein aggregation diseases like Alzheimer's and Huntington's disease visualized in terms of the FoldFx model. HSF1 signaling expands the proteostasis boundary, allowing it to recognize the aggregation-prone protein (*black node*) and alleviating the gain of toxic function. In contrast, FOXO-based pathways promote a protective form of aggregation that yields less toxic aggregates. The detoxified aggregate is indicated in the plot on the left side of the figure by the white node. As in **Figure 10**, the assigned values for folding stability and kinetics for intrinsically disordered proteins are meant to suggest the partitioning between states that are either susceptible to degradation or not.

polyQ expansions further sensitizes the organism to polyQ proteotoxicity (50), presumably as a consequence of consumption of proteostasis network capacity. This result illustrates the point that the proteostasis boundary for any one protein is dependent on the overall burden placed on the proteostasis network by other proteins. It also leads us to propose that any genetic predisposition toward protein misfolding/aggregation and/or disabling a component(s) of the proteostasis network could contribute to the sporadic forms of neurodegenerative diseases like Huntington's, Alzheimer's, Parkinson's diseases, and amyotrophic lateral sclerosis (ALS), simply by placing an additional load on the proteostasis network (50, 153).

Given the FoldFx model, it is apparent that aggregate load in neurodegenerative disease could be adjusted using either PCs or PRs. One approach to reduce aggregate accumulation is to utilize kinetic stabilizers, as described above, to stabilize the native non-amyloidogenic state of a given aggregation-prone protein, such as transthyretin. Another approach is to utilize small molecules that inhibit the aggregation of proteins at the self-assembly stage, such as 4,5-dianilinophthalimide (154), phenolsulfonphthalein (155), and others that appear to prevent the formation of mature (fibrillar) aggregates from nascent aggregates (156, 157). A potential limitation of aggregation inhibitors is that they may lead to the generation of smaller, more toxic oligomers, challenging the protective aggregation-promoting pathways of the proteostasis network.

Other strategies for using PRs to ameliorate neurodegenerative diseases beyond HSF1 or FOXO activation include overexpression of HDAC6, which has been shown to rescue neurodegeneration by way of the ubiquitin-proteasome system (UPS) and autophagy pathways (158), and use of HDACi to protect against Parkinson's (159) and Huntington's diseases (160). These observations suggest that these compounds may have PR activities that are linked to epigenetic features controlling the expression of degradative and aging-associated signaling pathways, thereby regulating the position of a node or the proteostasis boundary. A second possibility is suggested by the etiology of Parkinson's disease. A mutation in parkin, an E3 ubiquitin ligase (161), is associated with an early-onset form of Parkinson's disease, suggesting that Parkinson's disease involves a defect in the UPS. Therefore, PRs that potentiate parkin activity could ameliorate Parkinson's disease by repositioning the proteostasis boundary to favor degradation in general (162). Finally, in ALS (163), mutation of superoxide dismutase 1 leads to accumulation of reactive oxygen species in both cell-autonomous (164) and cell-nonautonomous (165) fashions that appear to contribute to protein misfolding, mitochondrial dysfunction, and oxidative damage, thereby challenging the proteostasis network. PRs that maintain the proteostasis boundary by dampening the impact of oxidative insult (163, 166) could be effective therapeutics for ALS.

The examples discussed illustrate only a few of the many ways in which the increasing evidence in the literature suggests that manipulation of the proteostasis network and the proteostasis boundary in a compartment- and cell-type-specific fashion with PRs may be a useful approach to expand proteostasis network capacity and mitigate disease progression.

THE FUTURE OF PHARMACOLOGIC MODULATION OF PROTEOSTASIS

The dependence of the function of distinct cell types on their proteostasis network capacities, and hence the position and shape of their proteostasis boundaries, is becoming increasingly evident. FoldFx describes a way to integrate folding energetics with proteostasis network capacity and provide a basis to think about adapting the proteostasis network for disease intervention. While the simplifications employed in our mathematical model undoubtedly mask additional approaches that may apply to a given cellular compartment or tissue environment, it can be expanded to account for more

complexity. It should be evident from this review that proteostasis is not as much about quality control as it is about using the combination of protein folding energetics and the adjustable proteostasis network capacity to optimally support function (45, 46). Because the proteostasis network and, hence, the proteostasis boundary are adjusted by signaling pathways such as the UPR or the HSR, these pathways mesh protein function with changing cellular and organismal needs (167–169). Given the fundamental interplay between folding energetics and proteostasis network capacity in maintaining the proteome, it is likely that numerous classes of compounds will be found that function as either PCs or PRs to ameliorate diseases of proteostasis deficiency. These new classes of pharmacologic agents (1, 170) should be widely applicable to the amelioration of human disease owing to the central roles that protein biogenesis, folding, maintenance, and degradation pathways play in physiology and pathology.

DISCLOSURE STATEMENT

R.I.M., A.D., and J.W.K. are cofounders, shareholders, and paid consultants for Proteostasis Therapeutics Inc. and FoldRx pharmaceuticals (J.W.K. only), which are developing drugs that could be PRs and PCs. W.E.B. is a shareholder in Proteostasis Therapeutics Inc. and a paid consultant for Proteostasis Therapeutics Inc. and FoldRx pharmaceuticals. E.T.P. receives milestone payments from FoldRx pharmaceuticals.

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