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Minireview

The proteostasis boundary in misfolding diseases of membrane traffic

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ABSTRACT

Protein function is regulated by the proteostasis network (PN) [Balch, W.E., Morimoto, R.I., Dillin, A. and Kelly, J.W. (2008) Adapting proteostasis for disease intervention. Science 319, 916–919], an integrated biological system that generates and protects the protein fold. The composition of the PN is regulated by signaling pathways including the unfolded protein response (UPR), the heat-shock response (HSR), the ubiquitin proteasome system (UPS) and epigenetic programs. Mismanagement of protein folding and function during membrane trafficking through the exocytic and endocytic pathways of eukaryotic cells by the PN is responsible for a wide range of diseases that include, among others, lysosomal storage diseases, myelination diseases, cystic fibrosis, systemic amyloidoses such as light chain myeloma, and neurodegenerative diseases including Alzheimer's. Toxicity from misfolding can be cell autonomous (affect the producing cell) or cell non-autonomous (affect a non-producing cell) or both, and have either a loss-of-function or gain-of-toxic function phenotype. Herein, we review the role of the PN and its regulatory transcriptional circuitry likely to be operational in managing the protein fold and function during membrane trafficking. We emphasize the enabling principle of a 'proteostasis boundary (PB)' [Powers, E.T., Morimoto, R.T., Dillin, A., Kelly, J.W., and Balch, W.E. (2009) Biochemical and chemical approaches to diseases of proteostasis deficiency. Annu. Rev. Biochem. 78, 959–991]. The PB is defined by the combined effects of the kinetics and thermodynamics of folding and the kinetics of misfolding, which are linked to the variable and adjustable PN capacity found different cell types. Differences in the PN account for the versatility of protein folding and function in health, and the cellular and tissue response to mutation and environmental challenges in disease. We discuss how manipulation of the folding energetics or the PB through metabolites and pharmacological intervention provides multiple routes for restoration of biological function in trafficking disease.
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1. Introduction

All organisms are locked into a limited set of protein folds from which function must be achieved. The chemical and energetic properties of the amino acid sequence of each polypeptide (the primary structure) dictated by the genome (DNA) and copied by RNA for the purposes of translation, provides only a basic 'score' that biology can read and interpret in multiple ways to evolve functionality through the activity of proteins. An understanding of how cells manage an initially unfolded ensemble of polypeptides, and generate and maintain the fold for function in human health remains to be understood. This is because, unlike folding in a dilute solution in the test tube, a protein in the eukaryotic cell is folded in many different crowded environments defined by the cell's compartmentalized organization that includes the cytosol, mitochondria, nucleus and the organelles comprising the exocytic and endocytic pathways. It is now essential to understand how the biological folding pathways within each of these compartments interpret the energetics of the protein fold and how it ultimately influences protein activity(s) to direct organismal health.

To generate a natively folded protein, a cell uses a large number of assistants to influence the acquisition, retention or removal of the fold. These assistants strongly influence trafficking through the exocytic (endoplasmic reticulum (ER), Golgi, cell surface) and endocytic pathways (cell surface, endosomes, lysosomes) that uniquely define the different eukaryotic cell and tissue biologies. These biological assistants regulate the protein fold and function. They comprise thousands of components that make up the protein homeostasis, or "proteostasis", network (PN) [1] and include well-established signaling pathways such as the unfolded protein response (UPR) [2–4], the heat-shock response (HSR) [5,6] and

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Ca^{2+}-sensing [7,8] and inflammatory pathways [9,10], collectively referred to herein as the folding response system (FRS). The FRS is complemented by the components of the ubiquitin-proteasome system (UPS) found in the cytosol, and lysosomal and autophagic degradation pathways collectively referred to as the degradation response system (DRS) [1]. The FRS and DRS operate as an integrated system to control the composition and capacity of variable folding environments in different cell-types. Both are highly responsive to physiological stress and changes in the metabolite load (the metabolome) that is strongly influenced by diet [11–13]. The PN is necessarily ancient and co-evolved with the remarkable diversity of polypeptide sequences and folds, very likely playing an instrumental role in expanding the capacity of polypeptides to function in complex cell and tissue environments, and to perform increasingly precise cellular tasks associated with both cytosolic and membrane compartments found in eukaryotes. Alterations in the polypeptide chain sequence (e.g., a mutation, co- and post-translational modifications), or as a consequence of a change in the concentration or the composition of the components of the PN, leads to a breakdown in the network resulting in human pathologies [14–16].

Herein, we focus on emerging evidence that a highly adaptive PN in each cell type can sense and respond to both normal physiology and a multiplicity of challenges to the protein fold for mobilization of protein cargo through the exocytic endomembrane trafficking pathway. We first describe a recently proposed global mechanism for how the PN may interpret and influence protein folding energetics to control the efficiency of folding based on the concept of a minimal 'proteostasis boundary' or 'PB' [17]. The PB is defined by the combined effects of the kinetics and thermodynamics of folding, and the kinetics of misfolding at a defined PN capacity of a particular cell type. Using the PB model as a framework, we explore what may go wrong in misfolding diseases, and discuss general dietary and pharmacological strategies that may be used to enhance proteome function to protect us from disease.

2. The proteostasis network

While small, single domain proteins can fold efficiently in the test tube, large, multi-domain proteins in the crowded environment of a cell often cannot. This circumstance dictates the need for the PN [1]. As noted above, the PN is an integrated biological system consisting of general and specialized chaperones, folding enzymes, degradation components, and regulatory pathways that control composition and concentration of the network components [5,18]. The proteostasis program is constantly challenged by changes in ATP, amino acid, and metabolite concentrations, ion balance, and physical insults including among others, temperature stress and pathogens. These not only alter the inherited capacity of the proteostasis program, but are sensed by the FRS and the DRS, which can either resolve the problem or promote cell death in the case of severe pathology [19–22]. The PN is controlled both cell autonomously and cell non-autonomously, the latter involving both neuronal and non-neuronal signaling pathways [23,24]. Thus, through transcriptional and post-translational mechanisms these pathways continuously monitor and balance the folding and function capacity by reducing protein synthesis, by enhancing folding and repair processes, and/or by mediating degradation.

While a core of PN components are highly conserved throughout evolution [23], the composition and capacity of the network is unique to each cell type [5]. Thus, any effort to understand in vivo protein folding will require consideration of the interdependence of folding energetics and the PN within a given cell type, and its response to the local tissue environment and organismal physiology [25,26].

3. Protein folding and energetics in biology and membrane traffic

An understanding of biological folding in the exocytic pathway necessarily involves integrating the energetics of protein folding with PN components. We have previously employed modeling to address this conceptual challenge [27,28]. Referred to as the FoldEx model [28], we described how the inherent energetics of the polypeptide chain are interpreted and influenced by the PN of the ER, the first step in a series of compartments required for the delivery of protein cargo to a multitude of post-ER exocytic and endocytic compartments, the cell surface, and the extracellular space. Using the Michaelis–Menten approximation to describe the kinetics of the PN components [28], we suggested that folding energetics and the capacity of individual pathways of the PN involving translation, chaperoning, degradation, and export together determine whether a given protein will fold and be exported from the ER, or be targeted for degradation (Fig. 1A).

More recently, the concepts embodied in the FoldEx model were extended to cover all situations where folding energetics and the PN work together to achieve and maintain biological function. Referred to as FoldFx [17], the role of the translation machinery, chaperones, and degradation pathways were more generally related to acquisition of 'function', an event that is analogous to the export step of the FoldEx model [28] (Fig. 1A). Like FoldEx, the FRS and DRS that regulate the composition and levels of proteostasis components are accommodated in the model by the adjustable concentrations of the PN components [17,28].

To appreciate the combined role of energetics and the PN in folding of all compartments of the cell we have proposed the concept of the PB [17] (Fig. 1B). The PB defines the minimal energetics that a protein must have to achieve folding and function in the context of a given PN capacity. The proteostasis boundary is best illustrated as a surface in a three-dimensional space defined by protein folding thermodynamics (from unstable to stable), folding kinetics (from slow to fast) and misfolding kinetics (from slow to fast) (Fig. 1B). Here, the variable disposition and shape of the PB is directly linked to the concentration of the proteostasis components and the metabolome of the cell. We display interacting proteins as a '*biological network*' defined by nodes (the proteins) and edges (their links to other proteins within the network) (Fig. 1B). Nodes are positioned according to their corresponding protein's folding energetics (their stabilities, folding rates, and misfolding rates). In a healthy cell, the position of each node and its edges relative to the PB indicates its relative stability in a given cell type reflecting the properties of the PN. Intriguingly, evidence suggests that the PN does not possess excess capacity, likely because maintaining capacity that is not immediately needed is a waste of cellular resources. Instead, the capacity of the PN may be adjusted to provide just enough capacity for the folding load in a given cell type at a given time [5,29,30]. We have suggested that by setting the proteostasis boundary as a threshold for folding and maintenance of the proteome [1], the network becomes sensitive to the local metabolome and can be adjusted in response to pathology to minimize damage and restore function. Thus, the PB can be thought of as a rheostat that can be turned up or down to adjust the folding capacity of a given cell type, ultimately achieving a level of functionality that dynamically drives human physiology and protects us from disease.

4. Folded versus unfolded in the eyes of the PB

Given the above, when is a protein folded or misfolded from an operational perspective? This is a challenging question, as up to 30% of proteins are now thought to have some level of intrinsic dis-

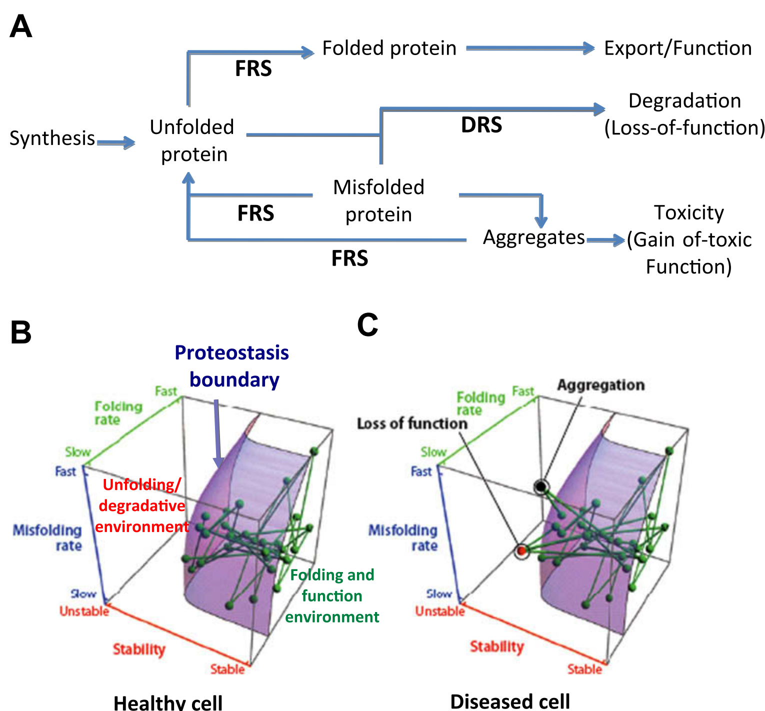

Fig. 1. The FoldEx and FoldFx models defining the proteostasis boundary. (A) Illustrated are the interactive pathways that require the FRS to either complete the fold for export from the ER (FoldEx model) [28] or for function (FoldFx model) [17], or contribute to misfolding, aggregation and degradation through the DRS. (B and C) Illustrated is the proteostasis boundary (PB) (indicated by the arrow) defined by kinetics of folding, kinetics of misfolding and thermodynamics reflecting a typical cellular composition of the PN (see [28] for details). The location of a hypothetical cellular network facilitating cell function is indicated by the green nodes and edges, which, in a healthy cell (B) is protected by the PN and therefore beneath the PB. In disease (C), a mutation can result in misfolding (red node and edge) leading to a loss-of-function disease, or aggregation (black node) that can lead to a gain-of-toxic function disease. See [17,28] for a thorough treatment of the impact of mutation on the PN leading to human diseases. Panels B and C are reproduced in a modified form from the Annual Review of Biochemistry, volume 78 [17] copyright 2009 by Annual Reviews, www.annualreviews.org.

order. This suggests that folding energetics may be defined and then redefined by the PN in a fashion that tremendously extends the initial role of the primary sequence encoded by the genome in a particular cell type. Moreover, how does the local environment, a mutation, or co- or post-translational modification alter a protein's ability to function in a biological network limited by the PB? In the case of mutation, we have suggested that a change in the amino acid sequence of a protein can significantly alter its folding energetics and its position relative to the inherited PB. As a consequence, this could place the protein outside the PB where it would become susceptible to substantial misfolding, aggregation, and/or degradation (Fig. 1C). The effect could be compounded if, for example, the protein was involved in interactions that stabilized other proteins resulting in ejection of multiple proteins from the protective embrace of the PB. In contrast, changes in the PN in disease, in response to diet and/or during aging could have a more global effect on the folding of the proteome, particularly the loss of core PN components such as heat-shock chaperones. Indeed, the insulin growth factor 1 receptor (IGF1-R), which regulates the expression of these components through heat-shock factor 1 (HSF-1), is now a well-recognized pathway that can be used to protect the cell against misfolding disease and prolong organismal longevity [5].

5. The proteostasis boundary in trafficking disease

Numerous diseases are a consequence of deficiencies in trafficking through the exocytic pathway and, in a number of cases, the function and stability of proteins in the endocytic pathway. The existence of compartmentalized function within the cell suggests that the cell actually operates in the context of multiple PBs that are unique for each compartment (Fig. 2). These compartments, particularly the ER and the Golgi, appear to be specialized to not only generate the fold (the ER), but also to post-translationally modify the fold (the Golgi) for downstream function in the cell, tissue and organismal environments. Indeed, the PB defined by the highly specialized ER proteostasis program (PB* in Fig. 2) is likely a 'master regulator' of folding for a given cell type given its rich chaperone content, its capacity to generate disulfide bonds [2,31] and its unique ability to add glycans that help stabilize the fold [32], although the underlying basis for its organization remains obscure. PB* clearly plays a primary role in defining not only the basic folding energetics during translation, but dictates both tissue and organismal physiology through generation of cell surface receptors and secreted proteins that communicate with other cells. While not fully understood at this juncture, the operation of the PN and configuration of the PB in a given compartment is intimately linked to the operation of the trafficking components (vesicle budding and fusion factors) that dictate the dynamics of exocytic and endocytic compartment function and PN composition [33].

Proteins housed in endomembrane compartments face different challenges. Soluble proteins that are temporarily or permanently found in the lumen of compartments are necessarily subject to that compartment's local PN (Fig. 2). On the other hand, transmembrane proteins are subject to multiple folding challenges-lumenal domains are subject to the PN of the local compartment. The cytosolic domain, although bathed in a 'constant' cytosolic environment, could be sensitive to PN components that are differentially tethered to the membranes of the different compartments in which they reside during their normal trafficking itinerary. The lumenal and cytosolic PNs must communicate with each other through unknown mechanisms. Moreover, cells generate protein cargo that must necessarily remain folded and function in the extracellular environment (Fig. 2). This suggests that unique PNs and PBs define

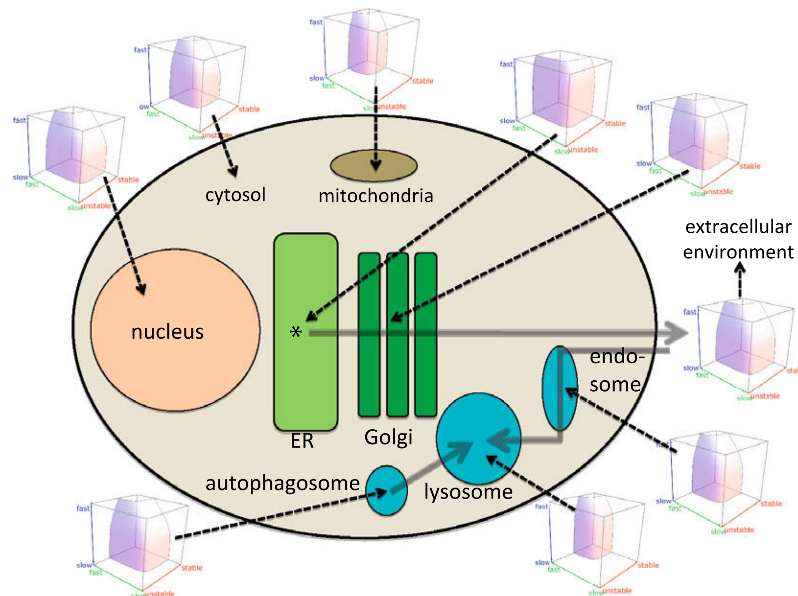

Fig. 2. Compartmentalization and proteostasis boundaries. Illustrated is a hypothetical view of the differing organizations of the PB found in the indicated compartments. The differences in the shape of the PBs reflect the differences in the PN present in each of these compartments or in the extracellular space that promotes folding and/or maintenance of the fold.

the normal operation of, for instance, the extracellular matrix surrounding each cell type in a given tissue and the functional composition of the blood plasma. Thus, as a consequence of compartmentalization in cellular physiology defined by the endomembrane systems, both local and distal PBs will have a major impact on protein function.

There are some features of the variant sequence (mutation) that are common to all membrane trafficking diseases that challenge the PN and hence the PB controlling normal cell, tissue, and organismal function. First, a missense or nonsense mutation can destabilize a protein, slow its folding, or accelerate its misfolding, such that the protein's folding energetics are no longer embraced by the PB (Fig. 1C). Such mutations can have different phenotypes. For example, mutations can trigger misfolding and degradation/aggregation in the ER. Alternatively, they may allow normal trafficking to the cell surface but rapid targeting for degradation by the lysosome through the endocytic pathway. Finally, they could result in a 'loss-of-function' (e.g., mutation of an active site residue in the case of enzymes), yet have normal 'residency' within the exocytic and endocytic compartments in the cell if the overall fold is not compromised. Second, trafficking diseases can place a wide range of demands on the PN. For example, expression levels can have a major impact on the PN by saturating folding and function capacity and/or lead to an imbalance of composition of multi-subunit complexes, triggering a more general disruption that challenges the capacity of the PN to maintain the PB at a given set-point. Thus, in considering the impact of protein folding energetics on the PB and human physiology, it becomes necessary to appreciate the fundamental basis for the folding problem in the context of the PN and multiple PBs defined by folding energetics that ultimately dictate function.

6. Taking care of business – membrane traffic and challenges to the PN

Unlike the cytosol, the nucleus or the mitochondrion, which have a 'captive' protein audience, the exocytic and endocytic pathways generate proteins for both local needs of the cell, and downstream needs reflecting tissue and/or organismal physiology. There are a minimum of four ways in which a misfolded protein in membrane trafficking pathways can be handled by the cell, tissue or organism that are discussed below.

The first possibility is that the variant is made, but is rapidly degraded because its energetics are outside the local PB in a particular cell type, leading to a loss-of-function phenotype. If the protein is essential for the producing cell, losing it would be detrimental to the cell, triggering death. We refer to this disease phenotype as cell autonomous toxicity (CAT). On the other hand, if the degraded protein is required at a site that is distal and largely independent of the producing cell's function, we to this refer to cell non-autonomous toxicity (CNAT). While many secreted proteins traversing the secretory pathway of, for instance, the liver or pancreas, are likely to fall in the CNAT category, transmembrane proteins are most likely to fall into CAT category as they are necessarily retained by the producing cell and often essential for growth, proliferation or function.

The second possibility is that the misfolded protein (either transmembrane or soluble) accumulates in the producing cell (e.g., it cannot be removed by the DRS). In this case, the accumulated/aggregated protein sets off a cascade of cellular responses to resolve the problem through FRS or DRS sensors that modulate the PN, resulting in a new protective PB. While this class leads to CAT unless resolved for both secreted and transmembrane proteins, it could also contribute to CNAT if, for instance, the aggregated protein trapped in the cell is not supplied at adequate levels at distal sites to perform a required function.

The third possibility is that the accumulated/aggregated protein (again, either secreted or transmembrane) exceeds the capacity of the FRS/DRS to mitigate the problem and therefore triggers CAT and cell death. This could also result in CNAT perturbation of both tissue and host function.

Finally, there is a pervasive class of disease in which the demand for production of even the wild-type protein exceeds the capacity of PN, chronically activating FRS and, where unsatisfied, cell death [3,10,34]. Because high expression can occur frequently during development, it remains possible that many developmental defects fall into this category and reflect the need for a specialized PN for each cell type during periods of rapid growth and differen-

tiation – a feature not unlike post-development cancers that are known to be sensitive to the PN [35,36]. In any event, it is the producing cell where the problem must be resolved. This may require adjustment of both the PN composition and hence the PB to protect the cell. Given the many mutations and polymorphisms that are found in a wide range of proteins, and their largely unknown impacts on the kinetics and/or thermodynamics of folding, it is not surprising that different mutations even within a single protein may trigger each of the distinct cellular responses indicated above.

Below we provide select examples of different classes of trafficking (mis)folding diseases and discuss their impact on the PN and the PB. In each case, we emphasize emerging approaches that could be used to augment the PN and its closely linked PB biology that could contribute to restoration of function.

7. Treating transmembrane trafficking disease

Cystic fibrosis (CF) is an inherited disease caused by mutations affecting the function of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel that regulates the hydration of the ductwork found in multiple tissues including the gall bladder, pancreas and intestine, and the surface of the lung [37]. To date >1500 disease causing mutations have been identified in the CFTR gene, suggesting that nearly every amino acid in the protein is important at some level. As might be expected, clinical presentation of these different mutations is very diverse, from mild to severe disease, reflecting the PN of the cell type in which it is expressed, the location of the mutation, and its impact on trafficking and function dictated by the compartment specific PB in the patient.

The most common mutation in CF disease is the Phe508 deletion (ΔF508 CFTR). This mutation disrupts folding and targets the protein for efficient degradation by the DRS in the ER, thus the energetic destabilization contributed by losing Phe 508 exceeds the folding capacity of the PB (Fig. 3). This conclusion is directly supported by the observation of a significant decrease in folding kinetics and thermodynamic stability of the mutant fold when compared to the wild-type CFTR [38]. Moreover, we have found that the activity of the PN components that contribute to the Hsp90 FRS system can either accelerate degradation, or protect either mutant or wild-type CFTR from the DRS depending on their levels in the cell [39]. Curiously, while stability in the ER can be improved by blocking the DRS [40], it does not necessarily result in export from the ER, suggesting that these events are uncoupled and respond to different features of the fold and the local PN. Not only does the ΔF508 phenotype fail to trigger known FRSs to facilitate 'correction', ΔF508 expression does not appear to interfere with cell proliferation or kill cells harboring the mutation (or lacking the protein in the case of the null). These results suggest that CF is best described as a CNAT disease. Indeed, it is the loss of tissue function that is pathological. This is strongly reflected in its coupling to sodium channel function, which together control cell surface hydration of multiple tissues [41].

Achieving correction in CF disease is a multi-faceted problem. First, we need to learn why the ΔF508-CFTR fold is unacceptable to the PN and PB found in the ER of most cell types, and determine if adjustments can be made pharmacologically that favor stability (Fig. 3). This could be accomplished by small molecules referred to as pharmacologic chaperones or 'correctors' [42], which directly bind to the misfolding-prone protein and provide additional stability as has been shown for G-protein coupled receptors (GPCRs) [43], Gaucher's [44,45] and transthyretin (TTR) mutants [46,47]. However, it is unlikely that simple pharmacologic correction of ER export is sufficient to solve the problem, given that the PN, and hence the PB controlling stability and function at the surface, is likely different from that of the ER. For example, the G551E

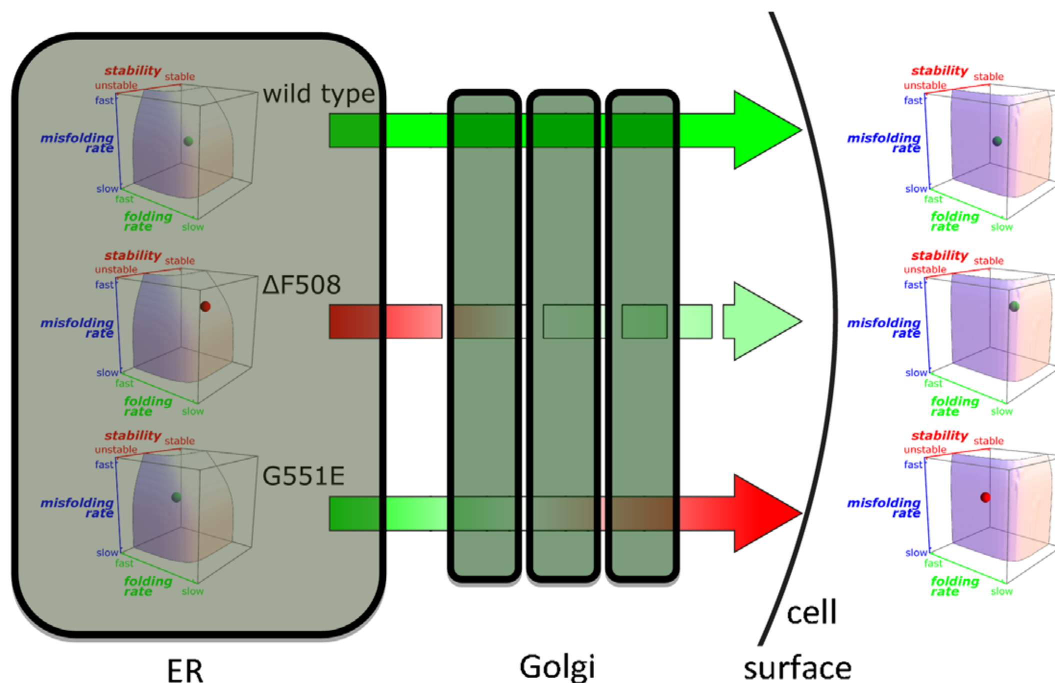

Fig. 3. Effect of the PB on stability of CFTR during folding and trafficking to the cell surface. Illustrated is the effect of two CFTR variants (ΔF508 and G551E) on trafficking through the exocytic pathway. Wild-type CFTR folds efficiently (indicated by the green) and is delivered to the cell surface where it has normal channel function (green). In contrast, the ΔF508 mutant is unstable in the ER (indicated by the red) and is efficiently degraded by the DRS. Addition of corrector or alteration of the folding environment by a proteostasis regulator may correct the folding problem in the ER and restore delivery to the cell surface where, given the proper environment, it may achieve a more normal level of channel function (light green). The G551E mutant normally folds efficiently in the ER (like WT) (green) and traffics to the cell surface, but has a defective channel (red) requiring either a potentiator (to activate the channel directly), or a proteostasis regulator that could affect the activity of the PN to improve open channel probability and hence, function.

mutation in CFTR has normal transport to the surface – yet lacks channel function leading to disease (Fig. 3). Here, a pharmacological chaperone referred to as a potentiator such as Vertex 770 (http://www.cff.org/research/ClinicalResearch/FAQs/VX-770/) could favorably alter (stabilize?) channel open probability, which is altered in ΔF508 [37], therefore artificially imposing functionality, as do agonists and antagonists for GPCRs [48]. Alternatively, correction could be achieved using proteostasis regulators [1], molecules that adjust the composition and/or concentration of the PN and hence the PB. The utility of proteostasis regulators in CF is supported by recent observations that correction can be achieved by adjusting the activity of Hsp90-dependent folding steps in the ER [39]. Moreover, a proteostasis regulator that adjusted the PB leading to folding and trafficking of the protein from the ER, could, in principle, provide a more stabilizing environment for function at the cell surface if the proteostasis regulator modified the kinase activities that regulate channel gating [37]. Of course, it is also possible that a combination of the various classes of pharmacologics (correctors, potentiators and proteostasis regulators) may synergize as has been observed for Gaucher's disease where rapid degradation could be reduced by proteostasis regulator, allowing sufficient protein to be produced to engage a corrector [45].

Although the above approaches may provide benefit, they beg the question of the real problem in CF disease and CNAT tissue dysfunction. It now may be important to define the signaling pathways that normally regulate surface hydration and thereby coordinate the cellular PN and CFTR function with the needs of the tissue and the host [41]. For example, by focusing on the more global regulatory circuits that control the PN responsible for tissue function through potentially unknown endocrine or neuroendocrine signaling pathways, as shown recently in *Caenorhabditis elegans* models of heat stress [23], such biologically coordinated integration of correction pathways may achieve an acceptable solution that will significantly benefit the patient. While we have focused on CF, it is simply one example of a large number transmembrane protein folding disorders where the variant fold is not essential for the producing cell type, yet essential for tissue and organismal physiology [49].

While CF is an example of transmembrane protein that, when mutated, is rapidly degraded, many mutations found in transmembrane proteins can challenge or even saturate the FRS and DRS of the producing cell, thereby triggering multiple stress responses within the cell and leading to CAT and cell death if not resolved. Examples of CAT include mutations in diseases of myelinating cells (including Charcot-Marie-Tooth disease, Pelizaeus-Merzbacher disease and Multiple Sclerosis) [50], diseases of connective tissues (ECM) [51], diseases of the eye including retinitis pigmentosa [52], mutations in the LDL-receptor that results in ER accumulation and activation of the FRS [53], and Alzheimer's disease resulting in the generation of extracellular and potentially intracellular toxic loads of amyloid challenging the local PBs [54,55]. All of these mutations and diseases challenge the PN and PB in different ways as the cell attempts to protect itself from the toxic consequences of what is a chronic challenge. CAT diseases need to be dealt with pharmacologically in a different way than CNAT diseases. Here, the challenge may not only be to restore functionality, but to abrogate the local toxic cellular load that triggers cell death. In principle, a corrector that adjusted the fold would solve the problem. However, that is a tall order for one compound – particularly when considering the chronic nature of inherited disease and the need for continual correction in response to the different PBs as the protein migrates through the exocytic and/or endocytic compartments, or is delivered to the extracellular space (Fig. 2). An alterative approach would be to make modest adjustments to the PN and the PB that mitigate the folding problem by modifying the activity and/or composition of the FRS or DRS. While it is true that such a strategy would be unlikely to be perfectly specific, since most FRS and DRS components have multiple clients, adjusting the PN and PB is likely to affect the levels of poorly behaved proteins (e.g., destabilized mutants) more than those of well behaved proteins. Furthermore, given that each protein, each mutation, each cell and tissue, and each local PN environment is likely to be unique, these compounds could show surprising specificity depending on concentration, dosing, and the FRS/DRS component targeted (e.g., E3 ligases are much more specific than the proteasome).

8. Treating secretory disease

A different group of misfolding diseases includes the many proteins that are synthesized as soluble proteins in the lumen of the ER and released by cells, particularly in tissues highly engaged in protein secretion such as the liver, pancreas, and the plasma cell. Like transmembrane proteins, these can fall into both the CAT and CNAT categories. An understanding of the effect of each mutation will be necessary for determining whether a disease should be treated by adjusting folding energetics using correctors, or by adjusting the PN using proteostasis regulators as suggested above. Known CAT/CNAT secretory diseases include, for example, α1AT deficiency [56], blood disorders of coagulation [57], congenital hyperthyroid goiter [58], procollagen disorders [59], and multiple systematic amyloidoses including light chain amyloid (AL) disease [60], gelsolin [61] and transthyretin (TTR) amyloid disease [46]. Many lysosomal storage diseases could also be considered 'secretory' diseases with the final destination being the lysosome and can be corrected by infusion of the missing soluble enzyme [62].

Gaucher's is an archetypal example of efficient removal of variant protein by the PN that invariably leads to loss-of-function and CAT. This class of disease can be corrected by pharmacologics that serve as correctors and/or that target the PN (8). In contrast, efficient degradation that is observed for certain α1AT variants synthesized by the liver do not directly affect the hepatocyte, yet loss of the protein leads to CNAT in the lung due to insufficient α1AT to counter the high protease activity required for normal lung function [56]. These could be corrected by folding stabilizers or by altering the PB controlling the targeting to DRS. On the other hand, as observed in congenital hyperthyroid goiter disease, and blood and bone disorders, the Z variant of α1AT can trigger aggregation in the ER which is normally cleared through autophagy pathways [56]. However, when aggregation exceeds the capacity of the autophagic system, it triggers severe liver disease and cancer [56]. In this case, targeting the misfolded protein for more efficient degradation to prevent aggregation or boosting autophagic pathways may be an effective first step in mitigating disease pathology, although the loss-of-function would be a confounding issue unless a balance between misfolding and production of some functional protein can also be met.

An example of a CNAT secretory disease that 'passes' one PB, but fails another is AL disease. This disease results in the generation of variant kappa or lambda chains by rare plasma cell populations [63]. Here, the light chain variant is sufficiently stable for synthesis and export. However, once secreted by the plasma cell, it is unstable in the extracellular environment in response to the different PBs affecting folding and maintenance in extracellular environments (Fig. 2) thereby triggering the formation of amyloid in distal tissues. For unknown reasons these variants fail to be recognized as 'problematic' by the ER PN. Here, it may be necessary to slightly increase the stringency of the PB in the ER in order to reduce variant trafficking and to promote degradation, thereby decreasing the levels found in the serum. Alternatively, it remains possible to alter the serum environment such that the variant fold is targeted for

degradation by the immune system. Similar problems lead to TTR amyloidosis [46] and gelsolin amyloidosis [61].

As a final example, it is not necessary have to a folding defect to challenge the PN, as even excessive expression of wild-type proteins in response to the metabolome can trigger disease as evident in type II diabetes [11,64–66]. Here, a high-fat diet, a lack of exercise, and genetic factors strongly impact the ability of the PN to maintain the function of β-cells in the pancreas in response to excessive insulin demand [4,11,67]. When the rate of insulin production exceeds the capacity of the ER-associated PN and PB to fold the protein, not only does insulin folding fail, but the PB collapses due to overload [17]. The collapse of proteostasis capacity in the β-cell is exemplified not only by extensive deposition of aggregates of the peptide hormone amylin that is synthesized along with insulin [68,69], but also by β-cell death, presumably resulting from sustained activity of the FRS. Strikingly, global modulators of the FRS, including the histone deacetylase inhibitors (HDACi) including 4-PBA [70,71] and activators of sirtuins [72–75] were found to attenuate the FRS response and restore β-cell homeostasis. Moreover, proteostasis regulators including the glucagon-like peptide-1 (GLP-1) receptor agonist exenatide [76] and inhibitors of dipeptidyl peptidase-IV that block GLP-1 degradation [77], may function by rebalancing the PN in the β-cell by globally attenuating endogenous FRS pathways at the organismal level. By readjusting the FRS using core signaling pathways, these proteostasis regulators effectively preserve insulin synthesis, protect β-cells from apoptosis and promote β-cell proliferation.

9. The next step in proteostasis disease management – learning to reprogram the PB?

While we have specifically focused on known membrane trafficking misfolding diseases, cancer cells clearly reprogram the PN to sustain proliferation [35,36]. Therapies that collapse this supportive PB and trigger cell death by increasing the folding load are currently in numerous clinical trials. Moreover, the success of viral, bacterial, and fungal pathogens may be far more dependent on the PN than previously anticipated given their need to exploit the PB for rapid propagation and/or survival [78]. While the dependence of the cell on the PN, and hence the position and shape of the PB, to integrate folding energetics with function in response to diet, stress and aging pathways is now evident, many challenges remain to learn how to reprogram the PB for benefit in the clinic.

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