Chapter 4
Managing the Adaptive Proteostatic Landscape: Restoring Resilience in Alpha-1 Antitrypsin Deficiency

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Abbreviations

PN	Proteostasis network
ER	Endoplasmic reticulum
AAT	Alpha-1 antitrypsin
Q-state	Quinary state
APL	Adaptive proteostatic landscape
AATD	Alpha-1 antitrypsin deficiency
NE	Neutrophil elastase
COPD	Chronic obstructive pulmonary disease
RCL	Reactive center loop
WT	Wild-type
COPII	Coatomer protein complex II
G/ERAD	Glycan ER-associated degradation
ER-phagy	ER-associated autophagy response
BAL	Bronchoalveolar lavage
HSR	Heat shock response
UPR	Unfolded protein response
MSR	Maladaptive stress response

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ERAF	ER-assisted folding and glycosylation
QC	Quality control
NEF	Nucleotide exchange factors
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor

Protein nascent chains emerging from the ribosome generate a native conformation through multiple folding pathways and diverse energetic intermediates, defined by a funnel-like folding energy landscape that shapes protein function in the cytosol and endomembrane trafficking pathways [1–7]. It is now recognized that "folded" proteins also dynamically fluctuate among different energetic states [8, 9] and ~30 % eukaryotic proteins can be disordered transiently or permanently at different times in their functional lifespan [10–13]. De novo folding and local conformational interconversions in vivo are particularly challenging to the cellular folding program and prone to misfold and aggregate in the complex intra- [12, 14] and extracellular [15–17] environments unless protected.

To address multiple folding challenges defined in the inherited genome, biology has evolved a sophisticated program, referred to as *p*roteostasis [5, 6, 18–25], which faithfully manages protein structure and the multiplicity of dynamic native conformations required to fulfill function in response to diverse signals stemming from complex cell, tissue, and organismal demands. This proteostasis program is an evolutionarily ancient system [19] that integrates protein synthesis, folding, degradation, and membrane trafficking systems including both the exocytic [endoplasmic reticulum (ER), Golgi, cell surface] and endocytic (endosomes, lysosome) pathways harboring alpha-1 antitrypsin (AAT) [26–28]. The proteostasis network (PN) generates a buffer that can be used to form a "cloud" around each energetic folding species that functions as capacitor to define the dynamic "*q*uinary state (Q-state)" of protein fold [19, 26, 29–31]. The Q-state encompasses the highly dynamic noncovalent ionic, hydrogen bonding, hydrophobic, and van der Waals surface forces responsible for all biology. It responds to reversible covalent modifications such as acetylation–deacetylation and ubiquitin modifications of surface Lys residues and facilitates integration of the more structurally ordered secondary, tertiary, and quaternary states encoded by the primary sequence to define the structural and functional properties of the protein fold. In response to Q-state properties, PN components can regulate the multiple conformations defining the energetic states of each folding species and also determine the turnover and intra-/extracellular location of each folding species. The PN contributes to a complex and dynamic in vivo structural and functional landscape of folding proteome, referred as the *a*daptive *p*roteostatic *l*andscape (APL) (Fig. 4.1). Modulating the composition, concentration, and/or function of PN components remodels the APL, hence regulating the function of folding proteome in response to folding stress—both acute and chronic as occurs in inherited or evolving environmental-triggered disease [6, 30, 32–36]. APL provides a novel

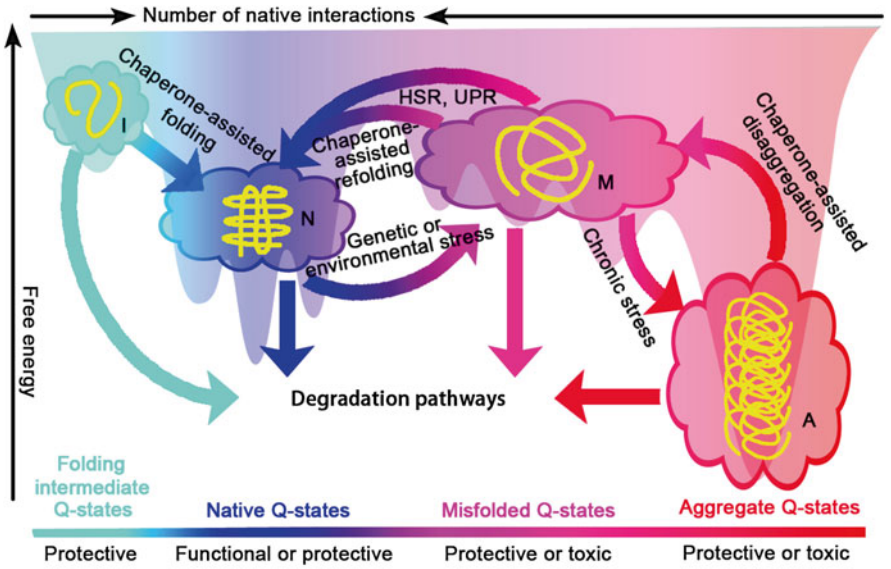

Fig. 4.1 A schematic diagram of APL. A protein adopts numerous energetic states illustrated here in a folding energy landscape with several states highlighted, such as folding intermediate (*I*), native state (*N*), misfolded state (*M*), and aggregate state (*A*). These states fluctuate among multiple local energy minimums. The PN recognizes these folding species harboring different thermodynamic and kinetic properties to define the Q-states and hence determines the functional environment (the Anfinsen physiological state) of each folding species. Here, a general "cloud" is used to represent the Q-state, but since the folding energy constantly changes, the Q-state likely also changes along the folding landscape, which is shown as a color scale from *cyan* to *red*. The conversion between Q-states and/or the turnover of folding species is highly dynamic and largely affected by both the PN and global stresses. Thus, modulating PN components could rebalance the APL from the stressed/diseased state to healthy state. HSR, heat shock response; UPR, unfolded protein response

therapeutic paradigm for all protein misfolding diseases [18, 20, 26, 30, 37] including *a*lpha-1 *a*ntitrypsin *d*eficiency (AATD) [23, 27, 38], which will be explored in this chapter.

AATD in the Context of Proteostasis Network

AATD is a consequence of point mutations produced in the inherited AAT gene [39–43]. AAT, which is mainly synthesized by hepatocytes, is the key antiprotease in serum that prevents the degradation of lung tissues by neutrophil elastase (NE) [40, 44–46]. The most common mutation triggering AATD is the Z-variant (Glu342Lys), which affects about 1 in 1600–3000 live births [47, 48]. We will primarily use this mutation to discuss the role of the PN in managing AATD, points which will equally apply to other AATD disease mutations [39, 40].

The Z-mutation alters the protein folding energetics of AAT. It produces misfolded and aggregated AAT that triggers serial responses from the PN, which differentially manages the APL in both the liver and lung in its attempt to maintain a healthy proteome for optimum biological activity [23, 27, 38], but sometimes fails [30]. When the capacity of PN is challenged in the liver, the increased polymerization/aggregation of misfolded AAT in the ER of hepatocytes leads to neonatal hepatitis [49], cirrhosis [50], and hepatocellular carcinoma [40, 51, 52]. On the other hand, the decreased pool of functional circulating plasma AAT [53] disrupts the protease–antiprotease balance in the lung and causes chronic obstructive pulmonary disease (COPD) and/or emphysema [27, 54–56].

In this review, we will first describe the proteostasis stress challenge in AATD and how the PN responds to the problem in the liver–plasma–lung axis. We will then discuss the rationale for therapeutically managing the APL by modulating the PN to mitigate folding stress and restore Z-AAT functionality and, finally, describe new potential therapeutic efforts by directly targeting the operational properties of the PN that are applicable to AATD.

The Folding Stress-Responsive APL in the Liver of AATD

Hepatocytes continuously synthesize and secrete a large amount of AAT to maintain a steady-state concentration range of 20–48 μM in serum [41]. To achieve a functional, folded form that can traverse the exocytic pathway, the newly synthesized AAT polypeptide chain needs to fold into a three-dimensional structure in the ER. Studies of the folding mechanisms of purified AAT in vitro revealed an intermediate folding state (*I*) between the unfolded state (*U*) and native state (*N*) (Fig. 4.2a), which is prone to misfold and polymerize [40–42, 57–62]. Furthermore, the native conformation of AAT is kinetically trapped in a metastable conformation with reactive center loop (RCL) transitioning through disordered and ordered conformational states during the AAT functional cycle [63, 64]. Therefore, the polymergenic intermediate state and the final metastable conformation render AAT with intrinsic misfolding and polymerization propensity—e.g., disorder. The Z-mutation (Glu342Lys) exacerbates this folding challenge seen for the wild-type (WT) AAT in the ER. Substitution of Glu342 by Lys slows down the folding rate of AAT [65] and deregulates the opening of β-sheet A, which leads to a misfolded intermediate (*M**) and an increasing tendency to form polymer (*P*) through a "loop-sheet" mechanism [40, 57, 58, 62, 66–68] (Fig. 4.2c). Additional models for AAT polymerization have also been proposed based on the crystal structures of a dimer of a serpin antithrombin [69] and a trimer of a disulfide mutant of AAT [70], suggesting that folding trajectories of AAT/Z-AAT could be diverse and likely dynamic, all subject to the local PN.

The PN has evolved specialized strategies for each energetic state of a folding species in order to maximize function. Abundant chaperones such as Grp78/BiP and Grp94/Hsp90 orthologs, calnexin, and folding enzymes [peptidylprolyl isomerase (FKBP) and protein disulfide isomerase (PDI) family members] in the ER, among

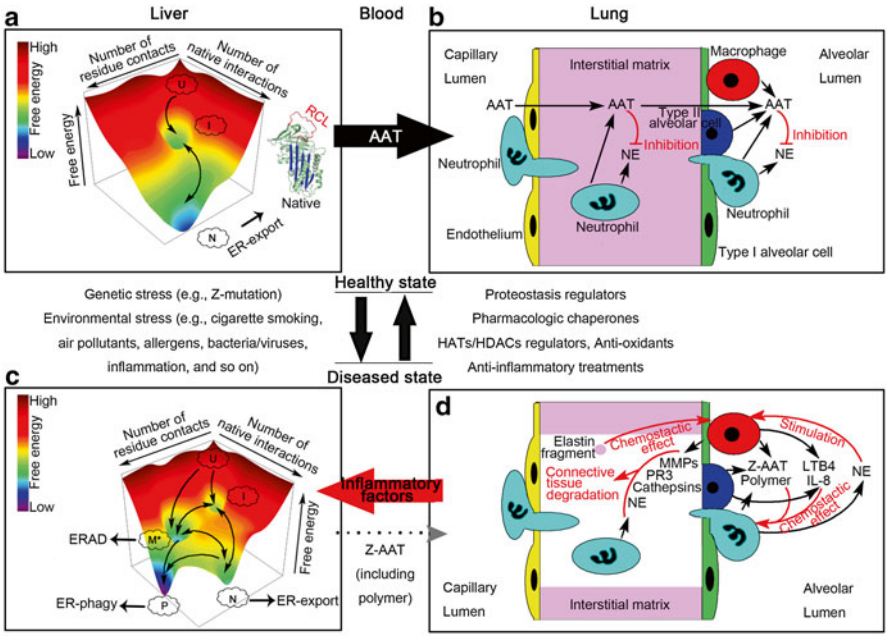

Fig. 4.2 The APL in the AATD context. (**a**) Newly synthesized AAT polypeptide (*U*) has high free energy and large entropy, which folds into native state (*N*) through intermediate state (*I*) in the ER and secrets into serum. Shown is the structure of the native conformation (1QLP) of AAT with reactive center loop (RCL) in red and β-sheet A in *blue*. (**b**) Abundant circulating AAT and locally secreted AAT inhibits NE to protect the integrity of lung tissue. (**c**) In ZZ-homozygous variants (or WT AAT in response to environmental stress), the folding of AAT may be trapped in a misfolded intermediate (*M**), which leads to accumulation of a polymer (*P*) in the ER. Here, the PN functions as a "cloud" surrounding each proteins folding state (the APL client) to help them traverse the free-energy barriers and/or sort different folding species to different destinies (i.e., lead the native folded monomeric state to the export pathway, a transiently misfolded intermediate to ERAD, and the polymer for degradation by the ER-phagy pathway). (**d**) Only 10–15 % Z-AAT is secreted into serum in AATD, which leads to continuous degradation of connective tissue by uninhibited NE, protease 3 (PR3), cathepsins, and metalloproteinases (MMPs). NE also stimulates the release of proteases (cathepsins and MMP-2) and chemotactic mediators (leukotriene B4 (LTB4) and IL-8) to accelerate lung damage. Digested elastin fragments and extracellular polymerization of Z-AAT contribute to lung disease through their chemotactic effect for monocytes and neutrophils, respectively. The trans-tissue inflammatory factors delivered from lung to liver exacerbate the folding challenge in the liver. We suggest that proteostasis regulators that modify the activity of the APL, chemical or pharmacologic chaperones that stabilize Z-AAT, HATs/HDACs regulators that adjust the Q-state and APL for a more productive folding event, as well as antioxidants and anti-inflammatory drugs, may provide therapeutic interventions to leverage the diseased APL back to a healthy APL and hence correct AATD

many others, prevent the aggregation of newly synthesized WT AAT polypeptides and help them reach a conformation that can be efficiently exported by the coatomer protein complex II (COPII) vesicle budding system [26, 71–79] (Fig. 4.2a). In contrast, because the folding of Z-AAT is extremely slow and thermodynamically compromised compared to WT AAT [65], the folding intermediates can be trapped

by the chaperones found in the ER [72, 80], a state which contributes to the increased concentration of intermediates susceptible to aggregation/polymerization—with only a small pool of Z-AAT apparently available secretion in its metastable state yielding a reduced serum load of 10–15 % that of WT.

We now appreciate that the ER PN has a set point that detects and partially targets the prolonged Z-variant intermediate for degradation through N-linked glycosylation pathways during its folding [75]. Here, polypeptides that cannot achieve sufficient thermodynamic stability in a biologically relevant time frame are processed by ER mannosidase (ERManI), removing several additional mannose units that typically occur in WT AAT processing for export. The energetics contributed by this processing event remains to be established. These intermediates are retrotranslocated to the cytosol for degradation by the proteasome through the glycan ER-associated degradation (G/ERAD) pathway [81–89]. In contrast, Z-AAT polymers/aggregates, which have been suggested in the past to be chaperome free (a point that requires further evaluation), cannot be degraded by G/ERAD pathway. One possibility is that the energy barrier for unfolding and/or retro-translocation across the ER membrane is too high. However, the PN has an alternative pathway to clear them by activating an ER-associated autophagy response (ER-phagy) [42, 43, 88, 90–96]. Therefore, these diverse folding species of Z-AAT with different biochemical/biophysical properties defining their Q-states are differentially recognized by the PN. This differential recognition defines their cellular fates and patient phenotype (Fig. 4.2c).

Remarkably, the ER APL is capable of resolving most proteostasis challenges in the liver brought on by the Z-mutation early in life, illustrating its robustness, since only about 8 % of ZZ-AAT homozygous newborns develop clinically significant liver disease [40, 42, 47, 97]. However, what changes during aging are the cumulative environmental and/or inherited/sporadic challenges leading to enhanced folding stress impacting APL function. This includes both genetic modifiers and environmental factors that either increase the Z-AAT folding challenge or decrease the proteostasis capacity [5] as well as the activity of the decaying stressed PN environment in response to disease [19, 30]. This contributes to late-stage liver and lung dysfunction characteristic of the aging AATD patient. Studies comparing the fibroblasts from “susceptible” and “protected” ZZ individuals reveal that a lag in degradation of translocated Z-AAT correlates with liver disease phenotype [40, 42, 82, 97], and a single nucleotide polymorphism that suppresses the translation of a key ERAD player, ERManI, was found to accelerate the onset of end-stage liver disease [98]. Thus, an increasingly impaired proteostasis program alters the APL and contributes to an early onset and/or a more severe form of the disease. It remains possible that the converse is also true—ZZ-AAT individuals with a more resilient PN may actually be protected and not be detected in the clinic until a very late stage of disease in response to either modifiers and/or management of the environment. It now becomes imperative to identify the role of the PN from the perspective of potential therapeutic targets reflecting the personalized proteostasis biology of the patient (see below).

AATD, Proteostasis, and Lung Disease

Unlike liver disease which can be detected in infants, the lung usually develops the first signs of AATD in early to late adulthood, suggesting that the increasing burden of misfolded/polymergenic Z-AAT derived from the liver and delivered via plasma to the lung generates an increasingly severe chronic PN challenge leading to pulmonary disease. Environmental pollutants (e.g., cigarette smoke) and pathogens (e.g., bacteria and viruses) that continually challenge the proteostasis program in the airway either directly through protein damage (e.g., oxidative modification) or indirectly through immune/inflammatory responses create additional stress that exacerbates onset of disease in the absence of protection by AAT [31]. For example, the risk of AATD lung disease is markedly increased by cigarette smoking, which can inactivate AAT (and other proteins [99]) through oxidation of the methionine residues in the RCL of AAT [99–103]. Moreover, cigarette smoking exacerbates the polymerization of both extracellular [104] and intracellular Z-AAT [105], decreases the transcytosis of circulating AAT from the capillary to alveoli [106], and upregulates the pro-inflammatory phenotype of lung cells that challenges the PN [105, 107]. Thus, cigarette smoking changes the structural/functional activity of AAT from multiple perspectives along the lung–plasma–lung axis to damage the proteostasis system of the lung independent of its impact on liver physiology, although increasing evidence suggests that cigarette smoke can contribute to liver disease.

The most prominent effect of the absence of AAT function in the lung is markedly increased NE activity leading to breakdown of the alveolar space. Specifically, inflammation in the alveolar wall induces the migration of neutrophils from alveolar capillary into the interstitium between the capillary endothelium and alveolar epithelium (Fig. 4.2b) [40, 42, 97, 108]. NE as well as protease 3 [109, 110], matrix metalloproteinases [111], and cysteinyl cathepsins [112] released from neutrophils degrade the lung interstitial matrix containing fibrillar collagens, elastic fibers, and proteoglycans and cause lung damage that leads to emphysema/COPD [23, 27, 113]. Although normally abundant circulating AAT enters the alveoli by transcytosis from circulating plasma [106, 114] to inhibit these proteases, local cells such as neutrophils [115], macrophages [116], and type II alveolar cells [117, 118] also produce AAT to protect the lung [40, 42, 97]. Thus, AAT functions as a component of a poorly understood PN network outside of the cell [17] to protect the integrity of the extracellular exposed lung proteome in response to inflammatory stress (Fig. 4.2b). However, in ZZ homozygotes, because the level of AAT in serum is only 10–15 % of normal healthy pool, this leaves the lung tissue subject to degradation by NE (Fig. 4.2d). Furthermore, because NE can stimulate the release of cathepsin B and matrix metalloprotease-2 from macrophages [119], this can exacerbate lung damage as the resulting degradation products are found to have a chemotactic effect for monocytes [120]. NE also upregulates the release of chemotactic mediators such as leukotriene B4 [121] and IL-8 [122], which leads to an increased concentration of neutrophils in the bronchoalveolar lavage (BAL) of AATD patients [40, 42, 97, 123]. Thus, the extracellular protease–antiprotease imbalance triggers a cascade of proteostasis mismanaged destruction of alveolar wall that causes defective alveoli (Fig. 4.2d).

Besides the loss-of-function mechanism, intracellular Z-AAT polymerization inside the monocytes [40, 67, 124] and/or lung epithelial cells [105] was shown to induce ER stress and release of inflammatory factors. Moreover, Z-AAT aggregates have been found in the BAL [125] and serum [53] of AATD patients. Given that polymer can be detected in patients that have undergone liver transplantation [126], these results potentially implicate a role of Z-AAT secreted from lung cells in polymer formation and accumulation in the lung. Some evidence suggests that extracellular polymerization of Z-AAT in the lung causes the influx of neutrophils into alveoli [126, 127] and further contributes to lung pathophysiology (Fig. 4.2d). Therefore, the misfolding/aggregation of Z-AAT is not restricted to its cell-autonomous impact on liver pathophysiology, but could play a prominent role in both cell-autonomous and cell-nonautonomous roles in the lung, confounding our understanding of the underlying proteostasis events responsible for the temporal (aging) and spatial (liver–plasma–lung axis) mitigation of disease.

Rationale for Managing the APL

The proteostasis problem is central for both liver and lung disease in AATD, so understanding the role of the PN is crucial for formulating new approaches for AATD disease intervention. The PN is a multilayered and fully integrated system composed of components that generate, maintain, regulate, and remove proteins [18, 20, 23, 26, 28, 30, 37]. As described in detail by Powers and colleagues [20], we define the innermost layer of the PN as the components that directly interact with and/or that affect the target protein including protein synthesis systems (transcription/translation machineries), folding/unfolding systems [chaperones and folding enzymes (the chaperome)], posttranslational modification systems (glycosylation, phosphorylation, acetylation and oxidation, among many others), trafficking systems (the exocytic, endocytic, and lysosomal pathways), and degradation systems including the ubiquitin–proteasome system (UPS) and the autophagy–lysosome axis managing the fate of cellular aggregates. The second layer of the PN is composed of multiple signaling pathways including the heat shock response (HSR), the unfolded protein response (UPR), and oxidative stress and inflammatory responses that modulate the composition, concentration, and function of the PN components comprising the innermost layer. These signaling pathways can trigger a cascade of events that promote cell/tissue health, reprogram the cell/tissue for repair or destruction, and/or support a malignant phenotype as found in neonatal liver disease [32, 128, 129]. The third, outermost layer includes environmental/physiological stressors, metabolites, and genetic/epigenetic factors that modify the first and second layer and are driven by the extracellular environment. Biology utilizes this sophisticated network to generally maintain the healthy state of the proteome that is susceptible to continuous genetic and environmental challenges, but is clearly challenged by the Z-variant and, for example, overwhelmed by a combined Z-mutation folding stress and an oxidative cigarette smoking-derived proteostasis

stress in AATD. To deal with the folding trajectory, it is now thought that the PN may recognize proteins based on their variable energetic standards contributing to a set point [19, 29, 30] that is sensitive to the unique proteostatic composition in each cell type and that is highly adaptable to the constantly changing stress that proteome misfolding places on proteostasis buffering (reserve) capacity. Moreover, recent studies show that proteostasis composition is under direct neuronal and neuroendocrine control [33, 130–132], leading to the recent suggestion that proteostasis cross talk between tissues, such as the liver–plasma–lung axis, could be integrated through the central and peripheral nervous system.

The above insights indicate that the conventional view that a single native protein conformation is "the" functional state is incorrect. It is likely that most proteins, particularly metastable AAT and its further destabilized Z-AAT variant, populate multiple intermediate functional states transiently associated with PN components throughout their lifetime, referred to as the "cloud" responsible for protein stability and evolvability [19, 35, 133]. Thus, it is the PN cloud that gives function to the dynamic Q-state of the protein fold in biology. In the case of WT AAT, while the cloud prepares the monomer to fulfill function as a NE inhibitor outside the cell, the polymer functions (in the context of the PN cloud) as a pro-inflammatory component that the tissue uses as a metric to call for support through immune responses. Besides the Z-mutation which dominates the current focus, there are over 100 different ATT mutations—more than 30 of which are found to affect either the level or function of AAT [134]. Interestingly, the thermodynamics of folding intermediates of most mutants (including Z-variant) are not dramatically different from that of WT AAT, but rather populate a series of modest free-energy wells in the folding energy landscape [135–137]. Where WT protein can easily traverse these energy wells with the help of the PN, AAT variants may become trapped and concentrated by local proteostasis cloud components because of either kinetic or thermodynamic problems contributed by the mutated Q-state structure (Fig. 4.2c). The fact that the diverse PNs in different cell types and compartments provide different levels of proteostatic capacity that are differentially sensitive to PN signaling pathways implies that the APL could be sculptable through reprogramming the PN [18, 20].

Z-AAT as a Sculptable Proteostatic Stress Modulator

Here, we use the folding of Z-AAT in the ER as an example to describe how the PN could modulate the APL and hence the disease phenotype. This description provides clues as to how we might leverage proteostasis to promote correction if we understand the mechanism(s) of action behind the interplay between the mutant protein fold and the local proteostasis cloud facilitating the folding trajectory.

While the tentative folding landscape of Z-AAT is shown in Fig. 4.2c, it is undoubtedly much more complicated. Each of the folding species dominating Z-AAT biology [e.g., *U* (unfolded nascent chain), *I* (on-pathway folding intermediate), *M** (off-pathway folding intermediate), *N* (native conformation), and *P* (polymer)]

is maintained by pathways that include PN-assisted ER translocation, ER folding and glycosylation [simplified as ER-associated folding (ERAF)] [19, 20, 138], degradation (ERAD, ER-phagy) [93], and ER export (COPII) [19, 138–141]. AAT is an acute-phase protein with its expression level increasing three- to fourfold in response to inflammation; thus, the ER import of *U* is highly variable and dependent on the signaling pathways dictating the functional needs of the cell, tissue. When the amount of *U* exceeds the capacity of ERAF that is critical to help stabilize metastable folding intermediates and prevent the aggregation of *U*, a process challenged by the thermodynamic/kinetic instability of all AAT mutants, the folding equilibrium of AAT, and particularly Z-AAT, will inevitably flow through the *M** and *P* pathways. Indeed, indomethacin, which activates IL-6-STAT3 cytokine signaling pathway and increases the expression level of Z-AAT, results in higher levels of Z-AAT polymers and increases liver damage in PiZZ mouse model [142]. Since Z-AAT has slow folding kinetics and low thermostability compared to WT AAT, the interconversion between *I*, *M**, and *N*, being concentration dependent, favors the conversion from *M** to *P* (or to a lesser extent from *N* to *P*). This process is unidirectional because once the polymer is formed, the stable conformation has an apparent large energy barrier for unfolding that the polymer cannot bypass [143, 144]. A solution that nature has evolved to remove such misfolded aggregates is to subcompartmentalize these thermodynamically stabilized structures in specialized, sealed degradation autophagic compartments [29, 43, 88, 90, 91]. This illustrates that by changing the APL through redefining the composition of the PN, the cell can flip the problem on its head to aggressively protect against aberrantly misfolded species.

The need for increased degradative capacitance to protect the cell from aggregated species is consistent with the observation that inhibiting ERAD without changing the capacity of ERAF and ER export increases the polymer level and contributes to AATD liver disease phenotype [82], reinforcing the interpretation that the dynamic folding equilibrium is under continuous control by the PN. Since our ultimate goal is to correct both liver and lung disease in AATD, we suggest that augmentation of ERAF and/or ER export machineries could increase the *I-N* folding pathway and decrease *M*-P* folding pathway, thus making the folding landscape of Z-AAT more like WT AAT as shown in Fig. 4.2a, possibly acutely or permanently correcting the fold by subtle changes in the APL [91, 145–147].

Proactive Proteostatic Signaling Pathways Activated by Z-AAT

The goal of proteostasis therapeutics is to use biology to fix biology by targeting the support pathways whose roots are 3.5 billion years old [19]. Remarkably, it is clear that biology, in response to the environment, has evolved many ways in phylogenetic diverse cell types to manage the fold and folding stress using common principles [19]. The versatility of the folding program should, in principle, be able to address challenges such as occurs in AATD-inherited disease through

reprogramming [18]. While HSR [3] is the universal response found to manage folding in all phyla, in eukaryotes the UPR [4] has evolved to handle misfolded proteins traversing endomembrane compartments. As such, each compartment relies on multiple but often unknown inputs to elicit highly variable and dynamic responses to handle folding challenges that either address the problem or found to be insurmountable and drive the cell down death pathways. Interestingly, Z-AAT does not activate the classic ER UPR signaling pathways that involve the IRE1, PERK, or ATF6 cytosolic transcription factors in hepatocytes [148–150]. The inability to generate an UPR response and promote complete degradation of misfolded Z-AAT through G/ERAD in hepatocytes could reflect the fact that the monomer and the polymer are actually considered as Q-state "normal" by the ER UPR signaling sensors, perhaps as a native but metastable "folded" structure that contributes to the dynamic "mouse-trap" mechanism [151]. However, the increased propensity to disorder in variants such as Z leads to a Q-state that largely defines a polymeric autophagic substrate and a modest substrate for G/ERAD.

In contrast to hepatocytes, Z-AAT induces UPR in monocytes where its expression is low [124]. Thus, in different proteostasis environments in which the aggregation phenotype may be reduced because of a smaller load of Z-AAT nascent chain whose polymer propensity is logarithmically proportional to concentration like amyloid, Z-AAT folding species may be sufficiently long-lived to be preferentially targeted to G/ERAD pathways [92]. These differences emphasize that diverse proteostatic dynamics found in different cell types can be targeted specifically by unique modifier therapeutics. Moreover, we have recently reported that the sustained expression of Z-AAT induces a maladaptive stress response (MSR) through chronic activation of the HSR pathway found in the cytosol [30]. A similar observation was reported in yeast where overexpression of a misfolded luminal protein CPY* was found to activate HSR [152]. Activation is unlikely due to UPR and/or altered G/ERAD because this phenomenon is observed in Ire1Δ strain in which both UPR and ERAD are impaired [152]. Importantly, we have shown that silencing HSF1, the HSR master regulator, either chemically or through the use of biologics (siRNA) restores the secretion of functional Z-AAT, indicating that the MSR confounds the disease phenotype in its attempt to solve it but cannot fix due to the chronic nature of the disease [30].

An additional proteostatic impact that has been noted in AATD is that the accumulation of Z-AAT activates oxidative stress [153] and inflammatory NF-κB pathways [27, 40, 148, 150] (or ER overload response [154]). How NF-κB pathway affects the PN is not well understood [27], but the inflammatory factors upregulated by this pathway, as well as trans-tissue inflammatory signals delivered from lung to liver (Fig. 4.2c, d), could increase the expression level Z-AAT, which may improve secretion but also could result in a higher production of polymer.

The above examples illustrate that proteostatic control in AATD could emanate from multiple and diverse signaling pathways reflecting the local cell and tissue PN response. A detailed understanding of the "personalized" folding of Z-AAT managed by proteostasis in these different cell, tissue, and host environments could

provide critical insights into unappreciated nodes in a correction network within the global PN impacting human disease.

Current Therapeutic Opportunities

AAT augmentation (replacement) therapy is the only specific noninvasive treatment for AATD [135, 155–159]. Replacement therapy is based on the concept that the patient should be able to restore the basal level of AAT by weekly intravenous purified human AAT, an approach that may slow or stop the progression of lung disease [135, 155–157, 160]. This treatment is indeed capable of contributing to the serum AAT pool above the protective threshold and to increase the anti-NE activity in BAL [155, 158, 161, 162]. However, the clinical impact of this treatment for lung function remains controversial [135, 156–159, 163–165]. The lack of a clear validated clinical impact by augmentation therapy could reflect the absence of well-controlled clinical trials to statistically assess apparent impact. Alternatively, the mechanism(s) responsible for lung disease could be much more complicated than just a shortage of circulating AAT below the protective threshold, perhaps reflecting the impact of the Z-AAT being produced by all cells (liver and lung), creating a proteostatic stress along the liver–plasma–lung axis by disrupting general proteostasis capacity and/or inhibiting the secretion of other unknown proteins/factors required for health. For example, issues such as acute-phase response and gain of toxicity of Z-AAT aggregates in the liver cannot be addressed by augmentation therapy. Moreover, the lung Z-AAT likely continues to create a burden that triggers inflammatory responses detrimental to lung health [134]. Furthermore, the continued production of Z-AAT could be a competitive inhibitor of perfused WT AAT function through as yet to be defined extracellular and transcellular proteostasis pathways [17, 19]. Environmental or genetic factors may also contribute to the efficacy of augmentation therapy from a personalized perspective that can only be resolved by understanding the steady-state dynamics of the APL in the liver, plasma, and lung and the impact of modifiers among the patient population.

In addition to augmentation therapy [135, 155–159], the liver and lung transplantation provide highly invasive but optional strategies for both early- and late-stage AAT-associated liver and lung disease [41, 166]. These treatments come with high risk and do not solve the global APL challenge from the perspective of continued Z-AAT production by other tissues. Progress on new therapeutic investigations reviewed elsewhere also comes from the perspective of gene therapy [167, 168], polymer inhibitors [40, 44, 53, 67, 68, 149, 169–172], and autophagy regulators [42, 43, 96, 146, 173, 174]. Below we will describe new opportunities for AATD drug discovery through proteostatic modulation of the APL.

Therapeutic Management Through the AATD Stress Chaperome

Chaperones (e.g., chaperonins, Hsp70, Hsp90 comprising the chaperome family) were initially described as proteins that assist non-covalent folding and assembly of polypeptides [2, 4, 14, 175, 176]. However, it is now recognized that chaperones also assist unfolding [177, 178], degradation [18, 21, 179–183], and trafficking [26] and direct the regulation of the biological function of thousands of client proteins including kinases driving cancer [128, 183, 184], DNA and RNA polymerase components [185, 186], transcription factors [187, 188], longevity factors [6, 189–191], most cell surface signaling receptors responsible for signaling proliferation (e.g., EGFR and steroid hormone receptors) responsible for metastatic disease [192], and cytoskeletal function [193, 194]. Chaperones also transmit the signal for misfolded proteins to activate UPR and HSR to manipulate the proteostasis capacity of the cell [4, 6, 18, 129]. Thus, chaperones provide the biological framework for regulation of the protein fold for function. They are potential key drug targets to manipulate both the dynamic Z-AAT folding Q-state and the signaling pathways controlling the PN that could alter folding dynamics to a more functional state to stabilize disease.

The major chaperones that bind Z-AAT in the ER include calnexin-UGGT system and GRP78/BiP (orthologue of the cytoplasmic Hsp70 family members) [4, 72, 80]. Through BiP, the secretory pathway is usually considered as a quality control system that only secretes the native conformer [22, 195, 196]. However, the concept of quality control is a misnomer. Rather than the native conformation per se, it is the global protein energetics and the variable PN that determine the success or failure of folding, export, and function and that is sensitive to genetic and epigenetic programs that define the physiology of each cell type [3, 30, 131, 139, 197, 198]. For Z-AAT, which is intrinsically metastable, competition for export and degradation components along the exocytic pathway will ultimately determine the final yield in the serum, emphasizing that it is not the AAT protein per se but the Q-state proteostatic cloud dominating trafficking pathways that dictates function driving liver and, ultimately, lung biology. While the ER export trafficking component ERGIC-53 has been suggested as a cargo receptor for WT AAT directing ER to Golgi exit, its interaction with Z-AAT is extremely low in the cell [44, 74, 199]. Multiple reasons could contribute to the low cargo receptor binding affinity for Z- compared to WT AAT including the following: (1) a large portion of Z-AAT is masked by the BiP/calnexin cycle; therefore, little Z-AAT is released for ERGIC-53 binding; (2) ERGIC-53 does not recognize the nonnative conformation of Z-AAT based on its thermodynamic or kinetic folding properties; and (3) the Z-AAT monomer–polymer balance in the ER is dominated by polymerization given its high concentration in the hepatocyte, an altered state that is not recognized efficiently by ERGIC-53. Thus, additional experiments are required to understand the underlying mechanism for ER export beyond the simple concept of quality control.

Other stress chaperones reported to interact with Z-AAT include GRP94 (orthologue of cytosolic Hsp90) [72, 200], GRP170 [72] and PDI [73], as well as numerous BiP and GRP94 co-chaperones—all poorly studied [40, 41, 201]. Collectively,

we refer to folding mediators as the ER "chaperome" that determines both the functional and degradative trajectory of the fold. Mutations in BiP lead to temporal impairment of ERAD of Z-AAT [179], and silencing of calnexin through siRNA increases the secretion of Z-AAT [202], which implies that these chaperones can alter the folding of Z-AAT in the context of their expression levels and the PN chaperome dynamics of a given cell type. This provides a simple demonstration that primary sequence, while important, provides a code that can be (re)interpreted by the chaperome/proteostasis system reflecting context.

The general mechanism used by chaperome biology is the dynamic binding and release of nonnative folding species to promote acquisition of the final folded state, presumably by preventing the formation of nonproductive, irreversible folding intermediate states (Figs. 4.1 and 4.2). Thus, it is not surprising that chaperones delay Z-AAT export from the ER since Z-AAT folds slowly and tends to misfold and therefore may reside more frequently in a "chaperone trap" by analogy to misfolded F508del CFTR [203]. However, the recognition that a substantial fraction of the Z-variant can escape the ER to yield a 10–15 % steady-state population in the plasma suggests that at a less populated state compared to WT AAT, Z-AAT occupies an energetically comparable folded state acceptable to the ER export machinery. Chaperones are also likely to be critical to manage the aggregation state of Z-AAT in the ER. However, no studies to date have addressed therapeutic management of Z-AAT through direct ER chaperome intervention.

What, then, could be the sequential events amenable to chaperome therapeutic intervention? We appreciate that the Z-AAT nascent chain enters the calnexin-UGGT cycle after co-translational removal of two glucose units by glucosidases I and II and then associates with BiP and ER export machinery components [75, 85]. ERAD is initiated when the binding of BiP reaches a peak. Thus, it seems that while the calnexin cycle is important for the initial folding and glycosylation of Z-AAT, BiP associates with Z-AAT in a late stage of folding and plays a central role in determining the balance of export, degradation, and aggregation. BiP alone is unlikely to be a direct drug target given its essential role for the folding of nearly all client proteins entering and/or exiting the ER and its critical role in controlling the UPR signaling pathway [4]. However, the function of BiP is regulated and diversified by its co-chaperone DNAJ proteins and nucleotide exchange factors (NEF) [72, 97, 204]. DNAJ proteins, which assist in client (AAT) delivery to BiP, often have distinct and specific client protein-binding properties due to their diverse structural organization in addition to the presence of the conserved J domain that interacts with BiP to promote function. After client protein binding, DNAJs recruit BiP, deliver the client protein, and stimulate BiP's ATPase activity to stabilize the client-BiP protein bound state. The release of the client protein from BiP is facilitated by NEFs (also called BAGs), which catalyze the exchange of ADP to ATP. There are seven DNAJ proteins and two NEFs that have been identified in the ER [205, 206]. However, there are few insights into their potential roles in WT and Z-AAT maturation. One NEF protein, GRP170, has been reported to interact with Z-AAT [72, 207, 208]. It would be interesting to see whether these co-chaperones play roles in determining the fate of Z-AAT delivery either to ERAD, aggregation, or export, since

targeting the binding interface between BiP and its co-chaperone and/or directly targeting co-chaperones is emerging as a new therapeutic opportunity. For example, targeting the cytosolic Hsp40-70 chaperome system for treatment of neurodegenerative disease and cancer is an active area of therapeutic development [2, 129, 181–183, 209, 210]. Consistent with this approach, we have suggested that elimination of chronic HSR (MSR) could restore the secretion of Z-AAT through MSR management [30]. These results point to the unanticipated direct or indirect importance of influence of the HSR on ER folding environment governed by the UPR and the cytosolic chaperome for modulating the APL of the Z-AAT. Though cytosolic chaperones are not directly involved in the folding of Z-AAT inside the ER, they have been shown to be critical for ERAD [211], autophagy [42, 91, 95, 96, 146, 212], and membrane trafficking [213]. Thus, it will be interesting to investigate how this proteostasis environment influences the general operation of the ER and downstream trafficking compartments in the management of Z-AAT proteotoxic stress in the liver and its function in the lung and whether they can be manipulated therapeutically through proteostasis regulators.

Therapeutic Management Through an Emergent Membrane Trafficking Proteostatic Code Driving Eukaryotic Biology

Numerous highly evolved endomembrane systems direct eukaryotic function [19, 26]. These provide optimal fold management systems reflecting the unique cellular, tissue, and organismal environmental challenges defining the APL. As such, trafficking has been proposed to be an extension of the proteostasis network [19, 26] with the trafficking machinery itself (the SNAREs, tethers, and vesicle-forming coats) defining the compartmentalized structure of folding pathways that facilitate delivery of protein to post-ER intracellular compartments [19, 26] and the extracellular space [17]. In the case of Z-AAT, folding species are exported out of the ER by COPII-coated vesicles [78, 214–218], which can mediate anterograde transport toward the Golgi and/or contribute to autophagosome formation [4, 42, 93, 94], divergent compartmentalization events impacting protein function [215, 219–221]. Exported material may be recycled back from Golgi compartments to the ER by the COPI coat system [78, 222, 223], refining the function of the ER folding environment and optimizing the composition of downstream Golgi and endocytic compartments [224]. The versatility of endomembrane trafficking design is consistent with the observation that Z-AAT can even exit the ER as a membrane-surrounded inclusion body, which is negative for autophagosomal and lysosomal markers, and can be inhibited by overexpression of calnexin [225]. Moreover, the ER has also been suggested to have COPII-independent mechanisms to dislocate large protein complexes involving the ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1) [226, 227], though the mechanisms remain unknown. Therefore, we consider membrane trafficking programs as dynamic modulators of the Anfinsen physiological state [26, 228], which utilizes developmentally specialized compartmentalization of

PN components to optimize use of the APL in each cell type, providing unique folding environments to respond to diverse proteostasis management signaling pathways that promote cell, tissue, and organismal survival.

Given the above, an untapped question is: how does the ER trafficking machinery recognize different energetic states of the incredibly diverse folding species exiting the ER to assess their specific destinations, such as autophagy, inclusion body, and/or ER-to-Golgi trafficking by COPII-dependent and/or COPII-independent mechanisms? Conventional ER export vesicles are coated by COPII complex, which have a typical size around 60–70 nm in diameter. Remarkably, we now appreciate that the size can be optimized depending on particular cargos, for example, collagen fibers (~300 nm) and lipoprotein particles (150–500 nm) require 300 nm vesicles [26, 215, 229–231] through the chaperome machinery. Cargo selection for export is initiated through the Sec23/24 adaptor complex that is subject to multiple posttranslational modifications for function [218, 232]. However, recent evidence suggests that the ability to generate diverse vesicle sizes that allow it to recruit large cargo involves additional trafficking components such as the adaptor escort proteins TANGO1 [233] and cTAGE5 [234] linked to the ER membrane. Moreover, it is sensitive to the ubiquitination state of one of the COPII components, Sec31 [26, 77, 229]. It will be interesting to see whether the size of COPII vesicles can be manipulated to differentially recruit WT and Z-AAT monomer or polymers for export as a therapeutic strategy. Even when Z-AAT successfully reaches in Golgi, resident proteins such as VIP36 and the KDEL receptor have been suggested to retrieve it back to the ER by interaction with its associated BiP [235]. VPS10 at the trans-Golgi has been shown to direct Z-AAT to lysosome for degradation [236], although it remains possible it has a normal function as an antiprotease inhibitor in the lysosome. Whereas considerable insight has been gained in understanding co-translational insertion and folding of the nascent chain in the ER, much less is known about the operation of the downstream trafficking compartments that could augment the protein fold through the COPI-mediated refinement and additional posttranslational modifications [78, 222, 223] that, when defective, are responsible for numerous pathological syndromes [26, 237]. Therefore, the unstable Z-AAT meets many challenges that can hinder its progress through exocytic pathway, highlighting the role of membrane compartments as proteostatic managers of the Q-state by generating sequential APLs. Additional studies may contribute to understanding the limitations restricting Z-AAT trafficking to serum for function in the lung.

Therapeutic Intervention Through the Acetylation–Deacetylation Network (HATs and HDACs)

We now appreciate that modifications of surface lysine (Lys) residues through competitive pathways of acetylation and ubiquitination can have a major impact on the Q-state responsible for protein stability and function by altering the response to the APL [19, 28–31]. The role of ubiquitination of surface Lys residues is complex and has been extensively reviewed [238]. Here, we focus on the impact of histone

acetyltransferase (HAT) and histone deacetylase (HDAC) in managing the APL in AATD.

HATs and HDACs mediate acetylation and deacetylation, respectively, modifications on the Lys residues of histones and nonhistone proteins. The acetylation status of a protein has a major impact on proteostasis pathways. While these modifications are well known to have an important role in the context of "epigenetics" that (re) program gene expression through histone modifications controlling nucleosome structure, we view a more general function of HATs and HDACs as "proteostatics" that modulate the charge status of surface Lys residues and, therefore, modify the Q-state for novel function in response to the local APL (Fig. 4.3). The importance of HAT/HDAC proteostatic activity is particularly evident not only in their impact in modulating posttranslationally the activity of many APL client proteins comprising the acetylome, but almost all PN components, including the core chaperones (Hsp90 [239], Hsp70 [240], Hsp40 [241], BiP [242, 243]), the HSR transcription factor HSF1 [244], degradation processes (by directly competing for Lys ubiquitination [245]), and membrane trafficking components including the assembly of microtubule and actin cytoskeletal polymers responsible for positioning and directing traffic through both the exocytic and endocytic pathways [246, 247]. Thus, HDACs globally impact proteostasis pathways by subtly modulating the thermodynamics and kinetics of the Q-state cloud biology in the context of the HDAC-

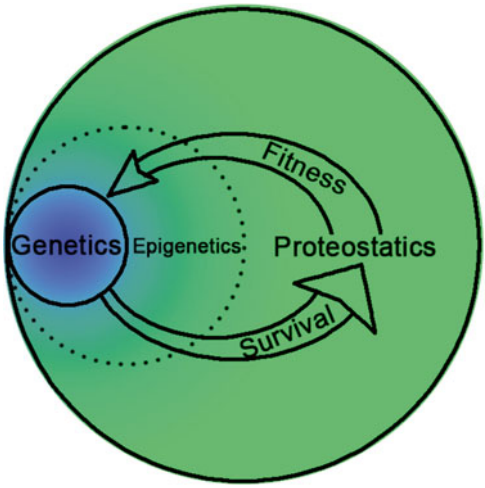

Fig. 4.3 The role of proteostatics in survival and fitness. Shown is the relationship between survival and fitness that is imprinted in genome through genetics, and epigenetic and proteostatic programs that are responsive to the environment. Genetics are defined by the inherited genome in the population that contributes to survival and fitness. Epigenetics, through histone modification of Lys residues by HATs/HDACs, alters the transcriptional environment of chromatin contributing to gene expression that promotes survival and "epi" (non-)genetic fitness in the population. We now appreciate that proteostatics encompasses not only the PN but includes epigenetics defined by HAT/HDAC-mediated acetylation–deacetylation pathways and contributes to the dynamic Q-state of the fold to promote daily survival in response to the environment and long-term fitness through modification of the genome

sensitive APL. In this view, proteostatic pathways that clearly encompass HDACs are potentially a therapeutic responsive real-time manager of protein folding imbalance in the cell [248] (Fig. 4.3).

Given the above, it is therefore not surprising that HDAC inhibitors (HDACi) have been shown to have beneficial effects for many protein misfolding diseases, such as cystic fibrosis [249], Gauchers disease [250], muscle atrophy [251], Niemann–Pick C [252, 253], and neurodegenerative diseases [254–258]. Specifically, we have shown that the pan Class I/II/IV HDACi suberoylanilide hydroxamic acid (SAHA) restores the secretion and activity of Z-AAT to ~50 % of the level that observed in WT AAT [27]. Currently, this correction appears to be mainly through inhibition of HDAC7, which is the same HDAC target that can be modified by SAHA to correct the folding of the most common mutation found in cystic fibrosis, F508del [249]. Incubation with SAHA or silencing of HDAC7 improves AATD through multiple pathways. For example, we have found that it upregulates the synthesis of Z-AAT, helps Z-AAT more efficiently exit calnexin chaperone cycle, modulates the ER redox environment by downregulating the expression of Ero1L, improves ER exit via COPII, and transits through the Golgi for improved secretion at the cell surface, as well as other pathways that we may not appreciate. This may simply be due to the fact that SAHA or silencing of HDAC7 produces a robust proteostatic encoded folding and trafficking environment (APL) for Z-AAT, which, interestingly, persists even after compound withdrawal following a chronic treatment regimen suggesting that imprinting occurs during epigenetic modification by HDACi [202]. Though SAHA-treated cells still produce polymeric Z-AAT, combining SAHA and polymer inhibitors [40, 169–171, 199, 259] may give an increasing yield of monomeric Z-AAT in plasma for lung protection.

In addition to its effect on Z-AAT folding and trafficking, SAHA exhibits anti-inflammatory properties (e.g., [260–264]), which could provide an unanticipated benefit in AATD by modulating inflammation in COPD [27]. However, the role of HDACs in lung biology is complicated. Class I and II HDAC activity is reduced in the lung of COPD patients even though the HAT activity remains unchanged [265, 266]. Moreover, reduction of HDAC2 causes corticosteroid resistance in COPD patients [267] leading to defective regeneration of airway epithelium [221], and reduction of HDAC3 increases the cytokine expression which would enhance inflammatory responses [268]. Besides the members of Class I and II, Zn^{2+}-dependent HDACs targeted by SAHA, SIRT1, one of the class III NAD (+)-dependent HDACs, is also reduced in lung of COPD patient [269]. Reduction of SIRT1 causes increased acetylation of histone H3 and NF-κB leading to upregulation metalloproteinase-9 expression affecting alveolar structure [270, 271]. In contrast, SIRT1 has been found to protect against emphysema by deacetylation of the FOXO3 transcription factor [272]. Consistent with these results, the SIRT1 activator resveratrol [262] and SRT1720 [273] were shown to alleviate lung inflammation.

In summary, by modulating the acetylation–deacetylation proteostatic balance driven by HAT-/HDAC-mediated Lys modification, it may be possible to both recover the shortage of functional antiprotease (AAT) production from the liver and decrease the threat from inflammatory pathways in the lung. However, it is clear that future

studies will be required to understand how the acetylation–deacetylation network managed by the HDAC population in the liver and lung tissue environments contribute to stabilization of function and to develop specific HDAC agonist/antagonists to optimize their impact on rescued Z-AAT in these environments. By using HDACi to alter the Q-state dictating protein folding stability and function, we may be able to synchronize the protein folding and function within the APL of multiple environments, an approach that could have an impact on disease etiology by using the same pathways biology nature has evolved to equilibrate the activity of diverse protein structures in a common proteostatics environment through Lys modification (Fig. 4.3).

Perspective: The Future of Proteostasis Management in AATD

We have described potential rationales and possible strategies to manage the APL and hence the function of the folding proteome responsible for human AATD. Though Anfinsen, who received the Nobel Prize in 1972 [228] for demonstrating that the primary sequence encoded by the genome plays a key role in generating the protein fold, he also posed a major challenge by suggesting that the biologically active conformation of a protein is actually determined by its environment—referred to as the physiological state. For the most part, the current practice of medicine ignores the underpinning dynamics of this physiological state as a focus for therapeutics. Given the evolutionary process [133], by definition we are all mutants. We achieve function as a consequence of proteostatic optimized evolvability [19, 34, 192, 274] that promotes daily survival and ultimately, through emergent proteostatics, fitness [19, 35, 275]. During the AAT functional cycle, the RCL needs to fully insert into the β-sheet A to flip the targeted elastase to another side of AAT [63, 64], which is likely thermodynamically and/or kinetically favored by the Z-variant mutation due to the increased metastability of the β-sheet A. However, this potential "functional" adjustment renders Z-variant polymerization propensity too great since the metastable β-sheet A also induces the intermolecular RCL insertion [40, 199, 276]. Such "off-pathway" mutations persist through evolutionary selection because of the highly evolved PN, which buffers these out-of-balance energetic states [19, 274]. Only when the normally robust PN is impaired in response to global stress or chronic aging does the "misfit" folding species begin to exhibit its proteotoxic function as occurs in AATD. We are now at the beginning of our efforts to remove the cloak masking the role of the PN in managing inherited genetic changes—proteostatic buffered changes that have worked in a positive sense and exceedingly well over the last 3.5 billion years to evolve and diversify extant life [19, 275]. We project that future studies defining proteostatic principles will provide insights and tools to adjust the dysfunctional APL found in AATD back to a healthy state, leading to reduced fragility and improved resilience of the liver and lung environments.

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