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Innovative strategies to treat protein misfolding in inborn errors of metabolism: pharmacological chaperones and proteostasis regulators

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Abstract To attain functionality, proteins must fold into their three-dimensional native state. The intracellular balance between protein synthesis, folding, and degradation is constantly challenged by genetic or environmental stress factors. In the last ten years, protein misfolding induced by missense mutations was demonstrated to be the seminal molecular mechanism in a constantly growing number of inborn errors of metabolism. In these cases, loss of protein function results from early degradation of missense-induced misfolded proteins. Increasing knowledge on the proteostasis network and the protein quality control system with distinct mechanisms in different compartments of the cell paved the way for the development of new treatment strategies for conformational diseases using small molecules. These comprise proteostasis regulators that enhance the capacity of the proteostasis network and pharmacological chaperones that specifically bind and rescue misfolded proteins by conformational stabilization. They can be used either alone or in combination, the latter to exploit synergistic effects. Many of these small molecule compounds currently undergo preclinical and clinical pharmaceutical development and two have been approved: saproterin dihydrochloride for the treatment of phenylketonuria and tafamidis for the treatment of transthyretin-related hereditary amyloidosis. Different technologies are exploited for the discovery of new small molecule compounds that belong to the still young class of pharmaceutical products discussed here. These compounds may in the near future improve existing treatment strategies or even offer a first-time treatment to patients suffering from nowadays-untreatable inborn errors of metabolism.

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Introduction

To maintain a healthy and functional proteome a living cell has to assure a correct balance between protein synthesis, folding, and degradation. This balance is constantly challenged either by genetic (e.g., mutations) or environmental stress factors. The crowded nature of the intracellular milieu adds to this difficult task as proteins have to attain correct folding in a place where concentrations of macromolecules can reach 300 mg/ml (Hutt and Balch 2013). Cells have evolved complex systems of molecular chaperone networks that help proteins to achieve their native structure from the translating ribosomes to post-translational folding. To attain functionality, proteins must fold into their three-dimensional native state structure during or following translation at the ribosomes (Kim et al 2013). While some proteins are able to fold spontaneously, most proteins, in particular large ones, are less efficient at folding and more vulnerable to misfolding (Kim et al 2013). Especially large proteins with complex structures tend to expose hydrophobic groups, rendering these susceptible to interactions that may lead to aggregation (Kim et al 2013). To avoid protein misfolding and consequently aggregation of proteins, multiple classes of chaperones are involved in the regulation of the folding mechanism, assisting de novo folding and maintaining the protein in its native state or regulating degradation of misfolded proteins. The balance

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between cellular protein folding and degradation is often referred to as protein quality control system and is one of many processes influencing protein homeostasis (proteostasis) (Balch et al 2008). Regulation of protein concentration, conformation, location, and degradation of individual proteins is the basis of the proteostasis network. The inability to restore proteostasis after a disturbance, for instance induced by a mutation, leads to diseases categorized as loss-of-function or gain-of-function disorders (Balch et al 2008).

In the last ten years, protein misfolding induced by missense mutations was demonstrated to be the seminal molecular mechanism in a constantly increasing number of inborn errors of metabolism. In these cases, loss of protein function results from early degradation of missense-induced misfolded proteins by the proteasome system or the lysosome (Ulloa-Aguirre et al 2004; Gregersen 2006; Gersting et al 2008; Mu et al 2008; Jorge-Finnigan et al 2010; Parenti et al 2013). Increasing knowledge on the proteostasis network and the protein quality control system has led to new treatment strategies for conformational diseases by small molecular weight (SMW) compounds (< 500 Da). These strategies comprise proteostasis regulators that enhance the proteostasis capacity and pharmacological chaperones that specifically bind and stabilize the conformation of proteins.

Herein, we aim to give a state of the art overview of the protein quality control system involved in folding and degradation of newly synthesized cytosolic proteins and on therapeutic protein stabilization. A deeper insight into the mechanisms involved in maintaining proteostasis has given rise to new therapeutic strategies for the treatment of an increasing number of inborn errors of metabolism associated with protein misfolding. Recent studies have shown promising results of proteostasis regulators and pharmacological chaperones alone or in combination to ameliorate enzyme function in inborn errors of metabolism by influencing the proteostasis network or by directly stabilizing target proteins by pharmacological chaperones (Mu et al 2008). This has offered the opportunity to specifically treat severe forms of inborn errors of metabolism and to replace current burdensome and sometimes ineffective therapies (Muntau and Gersting 2010).

The mechanics of protein folding and misfolding and the protein quality control system

The linear sequence of the polypeptide chain contains all necessary information to specify a protein's three-dimensional structure (Anfinsen 1973). Translation of this linear sequence into the functional protein is a slow process in the eukaryotic cell, where the polypeptide chain, based on an mRNA template, is assembled at the ribosome (Vabulas et al 2010). Thereby nascent chains are exposed in partially folded, aggregation-sensitive states. In addition, newly synthesized polypeptides must be transported into subcellular compartments, such as the endoplasmic reticulum or the mitochondria prior to folding (Kim et al 2013). These partially folded or misfolded states often tend to expose stretches of hydrophobic amino acid residues and regions of unstructured polypeptide backbone that in the native protein are buried from the solvent (Hartl and Hayer-Hartl 2009; Vabulas et al 2010). Furthermore, due to the vectorial nature of the translation process, complete folding can only occur once a complete protein or at least a domain has emerged from the ribosome. Thus, folding does not occur in a single-step manner but with various conformations in a folding energy landscape (Fig. 1) representing various intermediate steps along the way toward the native-state. Energetically favorable conformations or misfolded intermediates are stabilized as the protein progresses on a downhill path through the energy landscape. Nonnative conformations can be kinetically trapped in low-energy wells. These misfolded intermediates require reorganization by molecular chaperones before the native-state can be reached (Vabulas et al 2010).

A large fraction of newly synthesized proteins require assistance to reach their folded native-state (Hartl 1996). The protein folding reaction proceeds under tight biological control exerted by a complex machinery, the protein quality control system. This system manages the pool of unfolded and partially folded conformations. Molecular chaperones are proteins that interact cotranslationally with the nascent polypeptide-chain or folding intermediates to stabilize or help a non-native protein to acquire its native conformation, inhibit premature misfolding and aggregation and produce a low-energy functional protein, but are not present in the final functional structure (Hartl 1996; Hartl and Hayer-Hartl 2009; Vabulas et al 2010). Different classes of structurally unrelated molecular chaperones have been described, often known as stress proteins or heat shock proteins (Hsp) (Vabulas et al 2010). Molecular chaperones are classified according to their molecular weight indicated in the name of the chaperone, Hsp40, Hsp60, Hsp70, Hsp90, Hsp100, whereas small heat shock proteins exhibit a molecular weight in the range between 15 and 40 kDa (Vabulas et al 2010). Folding chaperones promote folding, while holding chaperones maintain solubility and unfolding chaperones disaggregate aggregates or unfold misfolded proteins and lead them to a new round of folding or to proteases for degradation (Gregersen et al 2006). These molecular chaperones are up-regulated by cells under conditions of conformational stress that lead to increasing concentrations of aggregation-prone conformational intermediates (Vabulas et al 2010). The chaperone pathways in the cytosol are highly conserved throughout evolution (Hartl and Hayer-Hartl 2002). First, ribosome-binding chaperones interact with the newly synthesized polypeptide chain. Secondly, chaperones without direct affinity to the ribosome assist in protein folding co- or posttranslationally after release of the

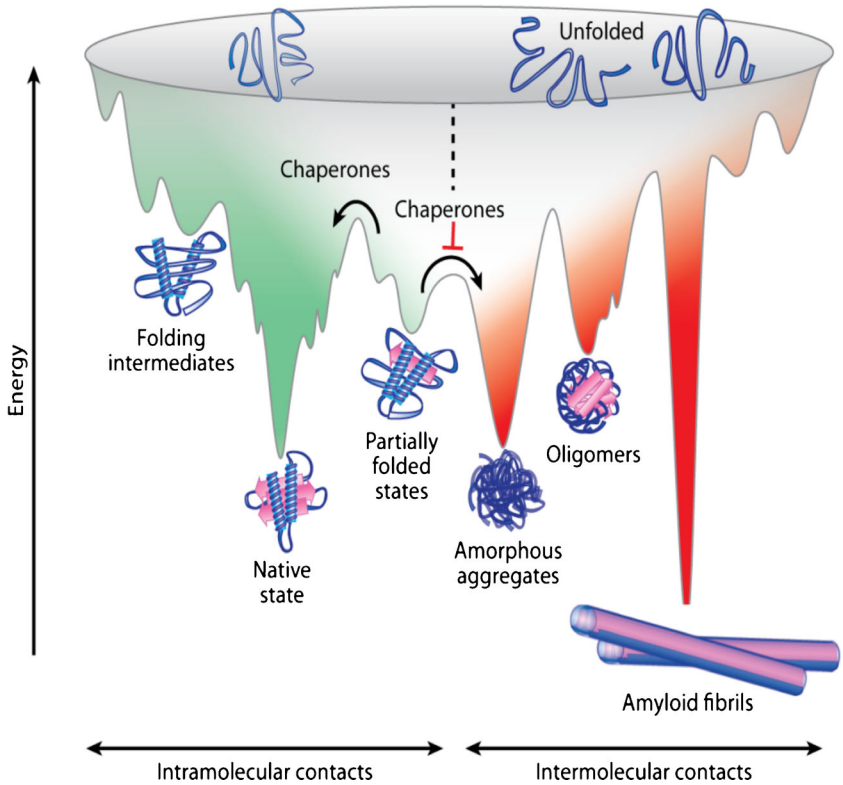

Fig. 1 Energy landscape of protein folding and misfolding. From the unfolded state toward the natively folded state, protein folding and protein aggregation are competing reactions. Energetically favorable *intra*-molecular interactions (green) are associated with an increase in conformational stability in the course of progression of protein folding downhill toward the native state. During this process, proteins may adopt energetically favorable but nonnative conformations that lead to populations of kinetically trapped states. These are located in low-energy wells (partially folded or misfolded states). Moreover, the kinetically trapped states are prone to establish *inter*molecular interactions (red), which result in protein aggregation (amorphous aggregates, β-sheet-rich oligomers, and amyloid fibrils). Chaperones assist in overcoming the free energy barriers, they inhibit intermolecular interactions and therefore promote folding to the native state conformation (Kim et al 2013)

protein from the ribosome (Kim et al 2013). Yet, these chaperones are not only involved in de novo folding of proteins but also in various aspects of proteome maintenance including macromolecular complex assembly, protein transport, degradation, aggregate dissociation, and refolding (Kim et al 2013).

Ribosome-associated chaperones

In eukaryotic cells ribosome-associated chaperones include the ribosome-associated complex (RAC), consisting of the Hsp70-like protein Ssz1, the Hsp70 cochaperone zuotin (Hsp40), and the nascent chain-associated complex (NAC), a heterodimeric complex of α- and β-subunits (Kim et al 2013; Leidig et al 2013). Their mechanism of action and their structure is not fully understood, yet these factors are abundant cellular proteins that may have additional functions in the cytosol or nucleus (Preissler and Deuerling 2012).

Ribosome-associated chaperones bind as soon as a polypeptide chain leaves the ribosomal exit tunnel. NAC has been described as the first chaperone to bind to the nascent polypeptide chain (Preissler and Deuerling 2012). This prevents aggregation of the protein while it is not yet fully synthesized. Due to the fact that the main folding process of the protein takes place after it is completely synthesized, the chain is vulnerable to unwanted interactions between exposed hydrophobic sequences and the solvent (Craig et al 2003). This mechanism is the initial defense against protein misfolding.

The Hsp70 family

This family of heat shock proteins can be found in all cells. These molecular chaperones have a central role in the cytosolic chaperone network (Kim et al 2013). The constitutively expressed heat-shock cognate proteins Hsc70 and the stress-inducible Hsp70 proteins of higher eukaryotes do not bind directly to the ribosome, but are involved in co- and post-translational assistance in folding of about 20 % of newly synthesized proteins. They are also implicated in many protein quality control functions such as refolding of stress denatured proteins, protein transport, membrane translocation, and proteolytic degradation of terminally misfolded proteins (Kim et al 2013). Hsp70 mediated folding follows kinetic partitioning with ATP-regulated cycles of substrate binding and release, where hydrophobic groups of newly synthesized

proteins are buried, hindering aggregation (Kim et al 2013). Hsp70 recognizes extended segments with hydrophobic amino acids, which are bound to the protein in an ATP-dependent manner. Hsp70s function in cooperation with Hsp40 or J-proteins and with various nucleotide exchange factors (NEFs) that regulate the reaction cycle (Vabulas et al 2010). The interaction of Hsp40 with Hsp70 strongly stimulates the hydrolysis of Hsp70-bound ATP to ADP, resulting in a closed conformation. Binding of NEF catalyzes the exchange of ADP to ATP, triggering substrate release (Kim et al 2013). Subsequent release of the protein allows for further folding of the protein. If several seconds are needed for complete folding, the protein is bound again to shield hydrophobic groups. Proteins that need a longer period of time or are unable to fold are transported to the downstream chaperonin or Hsp90 systems for folding (Vabulas et al 2010).

The Hsp90 family

Structurally, Hsp90 chaperones belong to the gyrase, histidine kinase and MutL superfamily of ATPases (Kim et al 2013). In eukaryotes, the ATP-dependent Hsp90 system assists in the final folding of signaling proteins or transcription factors, transferred from Hsp70. Substrate transfer from Hsp70 to Hsp90 is mediated by the Hsp90 organizing protein (HOP), which uses multiple tetracopeptide repeat (TPR) domains to bridge Hsp70 and Hsp90 (Young et al 2001). The Hsp90 system is very complex in its structure and function, and is to date not fully understood. However, recent work demonstrated that inhibition of histone deacetylases leads to decreased binding of Hsp90 to misfolded proteins resulting in protection of these proteins from early degradation (Yang et al 2013). In a broader context, Hsp90 is involved in a large variety of cellular processes including steroid signaling, calcium signaling, protein trafficking, vesicular transport, the cell cycle and cell division, immune response and the heat shock response (HSR) (Vabulas et al 2010; Kim et al 2013).

The sHSP

The small HSP (sHSP) chaperone system consists of many different heat-inducible, passive, energy-independent molecular chaperones (Haslbeck et al 2005; McHaourab et al 2009). These show pronounced structural plasticity and high affinity toward non-native proteins. These holding chaperones bind to unfolded proteins and by this prevent their aggregation. It is currently assumed that sHSPs function as storage depots for partially folded proteins that can be refolded by other chaperone systems upon their release (Richter et al 2010).

Chaperonins

Chaperonins are large double-ring complexes of approximately seven to nine 60 kDa subunits per ring with a central cavity, where substrate proteins are encapsulated one molecule at a time, which permits protein molecules to fold in an isolated compartment (Bukau and Horwich 1998; Kim et al 2013). Two groups of eukaryotic chaperonins can be distinguished. Group I chaperonins (Hsp60s) occur in mitochondria. They interact with the lid-shaped cochaperones Hsp10 to encapsulate substrates (Kim et al 2013). Group II chaperonins consist of the tailless complex polypeptide-1 (TCP-1) ring complex (TRiC) also known as chaperonin containing TCP-1 (CCT) (Kim et al 2013). Chaperonins act by global encapsulation and ATP-dependent cycles of folding. Hsp70 cooperates in transferring substrates to TRiC but also in cotranslational folding of nascent-polypeptide chains. A direct interaction between Hsp70 and TRiC has been shown (Cuellar et al 2008). Furthermore, group II chaperonins act with the co-chaperone prefoldin, which also transfers substrates to chaperonins and participates in the folding of actin and tubulin (Ohtaki et al 2010). Some substrates of TRiC are 100–120 kDa in size and therefore TRiC may assist in folding of single domains of larger proteins. In general, substrates of TRiC share no similarities in sequence (Vabulas et al 2010).

The proteostasis network

The term proteostasis describes the state of healthy proteome balance, whereas proteostasis network refers to the collection of cellular components involved in proteostasis maintenance. The proteostasis network integrates chaperone pathways for de novo folding, the conformational maintenance and disaggregation of misfolded states and protein degradation. The molecular chaperone network is central to the protein quality control system. It maintains protein conformation, reduces aggregation and guides misfolded proteins to degradation by the ubiquitin-proteasome system or autophagy. Molecular chaperones are thus the major regulators of the proteostasis network (Mannini et al 2012). The proteostasis network responds to different forms of cellular stress including the cytosolic heat shock response (HSR), the unfolded protein response (UPR) upon endoplasmic reticulum stress and the mitochondrial UPR. Furthermore, the proteostasis network is interconnected with pathways of inflammation, response to oxidative stress, caloric restriction or starvation and longevity. The proteostasis network of mammalian cells consists of approximately 1300 different proteins involved in protein biogenesis, conformational maintenance, and degradation. Every cell type possesses its own proteostasis network thus differing in proteostasis capacity, stress sensitivity, and vulnerability to protein aggregation (Kim et al 2013).

Protein misfolding in inborn errors of metabolism

Conditions associated with a cellular phenotype governed by misfolding of individual proteins or a disturbed proteostasis in general are referred to as conformational diseases or protein misfolding diseases. Protein misfolding follows two different pathophysiologic pathways, (i) protein misfolding with gain of function due to toxic aggregates, exemplified by classic neurodegenerative diseases such as Alzheimer's, Huntington's or Parkinson's disease; (ii) protein misfolding with loss of function due to inefficient folding and/or accelerated degradation or aggregation of proteins affected by genetic variation. In the latter group of disorders, missense mutations typically lead to reduced folding efficiency, reduced proteolytic and thermal stability, accelerated protein degradation and kinetics of aggregation, resulting in a loss-of-function phenotype (Muntau and Gersting 2010). Notably, the mutation-induced replacement of a single amino acid side chain in a polypeptide chain of typically several hundreds of amino acids suffices to hinder adoption of the protein's native state conformation. As shown in Fig. 2 for phenylalanine hydroxylase deficient in phenylketonuria, peroxisomal alanine: glyoxylate aminotransferase deficient in primary hyperoxaluria, and lysosomal glucosylceramidase deficient in Gaucher disease, amino acid side chains associated with protein misfolding molecular phenotypes can map to virtually all positions within the 3D structure of a protein. This notion elucidates the narrow spatial limits of native state conformations inside a protein that can sustain function and stability. Appendix summarizes a number of genetic diseases for which the molecular phenotype of protein misfolding was experimentally demonstrated and for which stabilizing small molecules have been identified. Inborn errors of metabolism predominantly belong to the group of protein misfolding diseases with a loss-of-function molecular phenotype (Muntau and Gersting 2010).

Treatment strategies for conformational diseases

The knowledge on pathophysiology and the molecular basis of genetic misfolding diseases caused by missense mutations is continuously increasing. This results in the identification of more and more candidate proteins for a pharmacological approach using small molecular weight compounds to pursue functional and structural rescue of misfolded proteins.

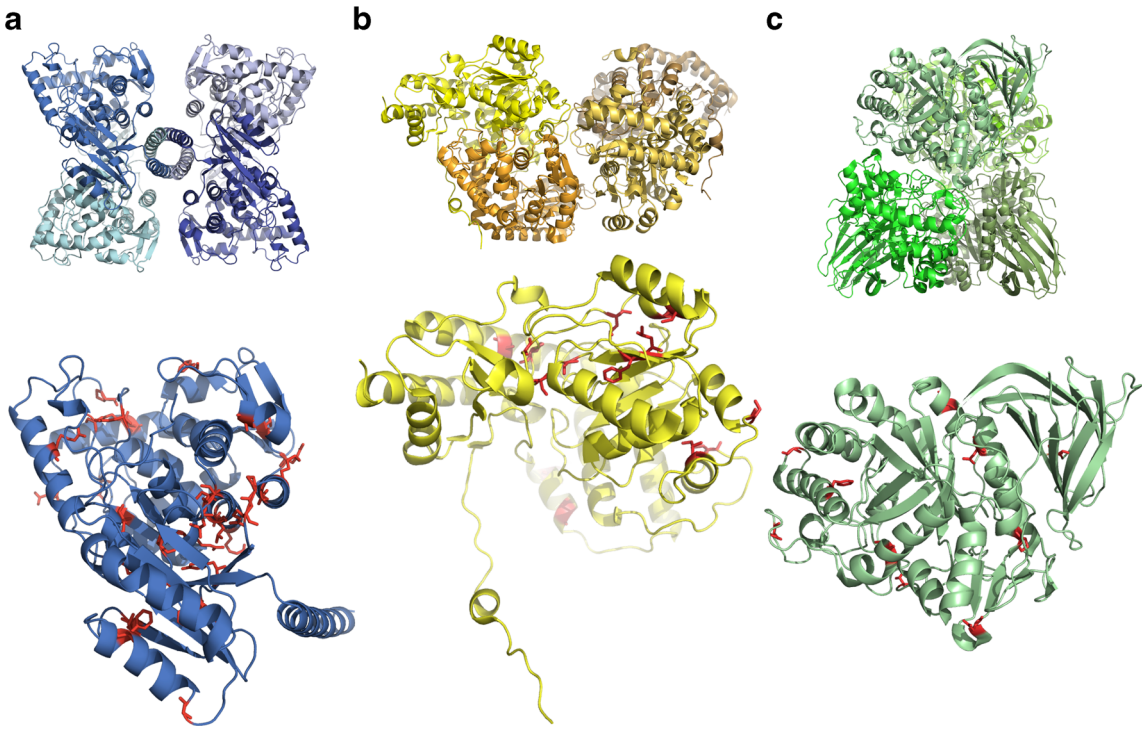

Fig. 2 Structural representation of proteins affected in conformational diseases and localization of side chains associated with protein misfolding molecular phenotypes. Missense mutations induce misfolding and loss of function of (**a**) cytosolic phenylalanine hydroxylase causing phenylketonuria, (**b**) peroxisomal alanine:glyoxylate aminotransferase causing primary hyperoxaluria, and (**c**) lysosomal glucosylceramidase causing Gaucher disease. The native state conformation of all three proteins shown here consists of a tetrameric assembly of identical subunits. Amino acid side chains affected by missense mutations and associated with a protein misfolding phenotype are indicated in red and can map to all parts of a subunit of the respective protein. Associations of side chain replacements with protein misfolding are taken from (**a**) Cerreto et al 2011; Erlandsen et al 2004; Gersting et al 2008; Leandro et al 2011; Nascimento et al 2008; Pey et al 2003, (**b**) Lumb and Danpure 2000; Mesa-Torres et al 2013; Oppici et al 2013; Williams and Rumsby 2007 (**c**) Edmunds 2010; Lin et al 2004; Mu et al 2008; Ron and Horowitz 2005; Schmitz et al 2005

Currently three different small molecule treatment strategies can be used to rehabilitate misfolded proteins and therefore restore mutant protein homeostasis in human misfolding diseases. The small molecular weight compound categories addressing these three strategies with different modes of action are chemical chaperones, pharmacological chaperones, and proteostasis regulators (Fig. 3).

Chemical chaperones are small molecular weight compounds that do not directly bind to misfolded proteins. Instead, they modify solvent conditions by changing the interaction capability of water molecules to interact with the protein, a hydration effect leading to high protein compactness (Singh et al 2007; Leandro and Gomes 2008; Arakawa et al 2011).

Pharmacological chaperones bind specifically to misfolded proteins, promote their conformational stabilization and by this prevent early degradation (Bernier et al 2004). The beneficial consequence from this is an increase of the effective intracellular protein concentration, that is the amount of functional protein in the cell.

Proteostasis regulators offer a generic approach to increase proteostasis capacity. They facilitate protein folding, enhance degradation of non-native protein species, and minimize misfolding. This happens by increasing the function and availability of molecular chaperones and/or by activation of the protein quality control system (Balch et al 2008; Mu et al 2008).

Chemical chaperones

The term chemical chaperone refers to compounds with a low molecular weight that stabilize the native conformation of proteins. This includes, e.g., polyols (glycerol and trehalose), amino acids (taurine and arginine) or methylamines (betaine and trimethylamine N-oxide). However, the therapeutic use of chemical chaperones in the treatment of misfolding diseases is limited. First, high concentrations are needed to achieve significant increases in protein function in vivo and such concentrations are often toxic (Arakawa et al 2006). Secondly, due to the underlying mode of action, chemical chaperones lack specificity and this may be associated with undesired effects (Underhaug et al 2012). Research dealing with chemical chaperones has been very useful for the understanding of general mechanisms concerning protein misfolding and stabilization of misfolded proteins. However, due to the limitations described above it is doubtful whether these compounds will ever play a role in clinical practice.

Pharmacological chaperones

Pharmacological chaperones specifically and reversibly bind to misfolded target proteins. This induces conformational stabilization of the latter consequently increasing the availability of functional protein species in the cell (Fig. 4) (Leandro and

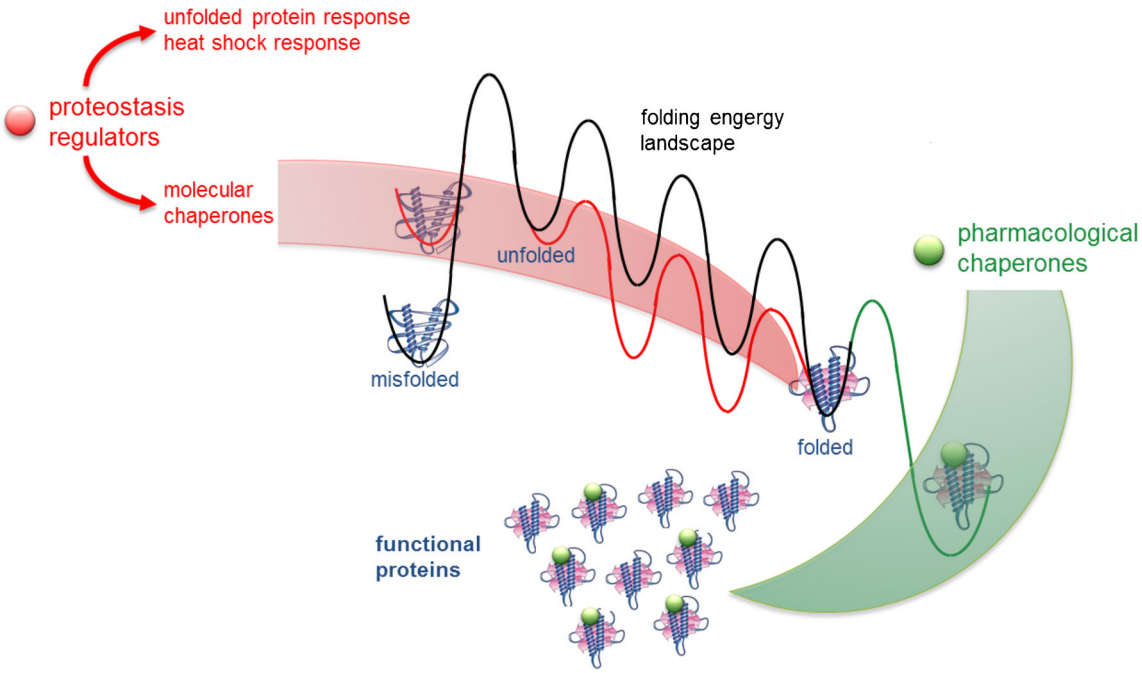

Fig. 3 Mode of action of proteostasis regulators and pharmacological chaperones. Both proteostasis regulators and pharmacological chaperones are able to restore mutant protein homeostasis by increasing the effective intracellular concentration of the functional protein conformation in loss-of-function disorders. Proteostasis regulators can activate the cellular protein quality control (e.g., unfolded protein response in the ER, heat sock response in the cytosol) to increase the availability of molecular chaperones. Proteostasis regulators can also directly enhance the function and activity of molecular chaperones. As a result, the protein folding capacity increases and protein misfolding is reduced (red arrow). Pharmacological chaperones specifically bind to target proteins and promote their conformational stabilization (green arrow). Both pharmacological strategies can be used alone or in combination as they show synergistic effects. Adapted from (Mu et al 2008)

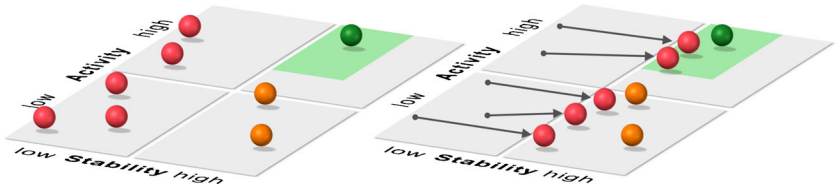

Fig. 4 Effect of pharmacological chaperones on function and availability of misfolded proteins. Protein misfolding induces states of lower stability and/or lower activity (red and orange spheres) as compared to the natively folded state of the wild-type (green sphere). Pharmacological chaperones bind to misfolded proteins resulting in increased conformational stability (grey arrows). In particular for proteins with high residual activity, binding of a pharmacological chaperone can induce a stability and activity level that corresponds to that of the wild-type protein (green area); but also for proteins with lower residual activity binding of a pharmacological chaperone induces an increase of the availability of protein species in the cell resulting in improved functional capacity of the affected metabolic pathway. Misfolded proteins associated with low enzyme activity but high stability may not benefit from further conformational stabilization by binding of a pharmacological chaperone (orange spheres)

Gomes 2008; Powers et al 2009; Muntau and Gersting 2010). This process is often associated with an inhibitory effect on protein function, which holds particularly true for enzymes (Fan 2008). Although a counterintuitive approach, inhibition-associated protein stabilization is a trade-off between a certain catalytic inhibition that is overruled by a significant increase in the availability of functional protein eventually rescuing the loss-of-function phenotype. General rules for small molecules acting as pharmacological chaperones are: (i) targets must have a mutation which renders the protein unstable but not inactive, (ii) pharmacological chaperones bind with high affinity and thus can be used at low concentrations, (iii) specific inhibitors work better than nonspecific stabilizers, (iv) pharmacological chaperones are effective if a small amount of the protein can make a big difference. Many natural ligands binding to the target proteins can act as pharmacological chaperones (Bernier et al 2004). These include protein cofactors, agonists and antagonists, competitive inhibitors, and ligands (Leandro and Gomes 2008). For membrane and secreted proteins pharmacologic chaperoning increases the concentration and proper localization of the protein by coupling more of the folded state to the trafficking pathway, a pathway that strongly influences proteostasis (Balch et al 2008).

Proteostasis regulators

The consequences of protein misfolding disorders with loss of function can be alleviated by boosting the intracellular proteostasis capacity (Kim et al 2013). This constitutes a much broader therapeutic approach than that using ligand compounds specifically stabilizing disease proteins. From a mechanistic point of view, proteostasis regulation in the context of human protein misfolding disorders with loss of function is linked to four functional intracellular principles. These are (i) regulation of protein production, (ii) chaperone upregulation, (iii) modification of chaperone function or (iv) enhanced degradation of misfolded proteins.

Guanabenz is a small molecule that induces attenuation of global protein production and by this reduces the client load for molecular chaperones (Tsaytler et al 2011). Numerous substances were shown to upregulate the production of molecular chaperones. Geldanamycin displaces HSF1 from Hsp90 converting HSF1 into a transcription factor for molecular chaperones. This has proven effective in several neurodegenerative disease models (Nagai et al 2010). The well-known antiepileptic drug carbamazepin modifies chaperone function. It strongly induces autophagy and thus enhances the clearance of potentially toxic proteins (Li et al 2013).

Modification of the ubiquitin proteasome system in the presence of protein misfolding can be beneficial in two different ways. Enhanced protein degradation can be achieved by increasing the activity of the ubiquitin proteasome system. This offers a strategy to reduce the levels of aberrant proteins in cells under proteotoxic stress (Lee et al 2010a). On the other hand, inhibition of the proteasome can prevent early degradation of still functional variant proteins. This was shown for Bortezomib, the first proteasome inhibitor approved, initially used for the treatment of multiple myeloma (Twombly 2003). Addition of the drug to cells expressing variant cystathionine β-synthase significantly increased the intracellular amount and function of the protein affected by a mutation (Singh et al 2010).

Regulation of histone deacetylases by specific inhibitors, first studied in the context of cancer therapeutics (Dokmanovic et al 2007), can have multiple effects on proteostasis at different levels. There is increasing evidence that histone deacetylases inhibitors (HDACi) also bear great potential for the treatment of protein misfolding disorders (Powers and Balch 2013). On the epigenetic level this class of drugs was proven effective to restore trafficking of mutant CFTR (Hutt et al 2010). Moreover, modulation of Hsp90 acetylation by the HDACi LB205 and SAHA induced an increase in quantity and activity of glucosylceramidase in Gaucher disease (Yang et al 2013). Pleiotropic effects were observed for 4-phenylbutyrate that provided benefit for numerous misfolding diseases including X-ALD, metabolic syndrome, cystic fibrosis, and α-1-antitrypsin deficiency (Kemp et al 1998; Powers et al 2009). This compound not only displays HDACi activity but additionally exerts a chemical chaperone effect that ameliorates protein misfolding (Cuadrado-Tejedor et al 2013).

Pharmacological chaperones and proteostasis regulators in clinical use or under investigation

Pioneering work in the field introducing the concept of pharmacological chaperoning was provided in 1999 (Fan et al 1999). Fan et al demonstrated that the imino sugar migalastat acts as a pharmacological chaperone in Fabry disease and rescues alpha-galactosidase A acitivity. From this time on many small molecules underwent preclinical and clinical trials. To date, only two pharmacological chaperones have been approved: sapropterin dihydrochloride for the treatment of phenylalanine hydroxylase deficiency and tafamidis for the treatment of transthyretin-related hereditary amyloidosis.

Compounds approved for the treatment of protein misfolding disorders

Sapropterin dihydrochloride for the treatment of phenylketonuria

Phenylketonuria (PKU; MIM 261600) is an autosomal recessive inborn error of the amino acid metabolism. The metabolic disease results in a reduction of the ability of the enzyme phenylalanine hydroxylase (PAH) to process the essential amino acid phenylalanine. The resulting increase in blood and tissue phenylalanine concentrations causes considerable damage. Symptoms of phenylketonuria are progressive mental retardation, brain damage, seizures, and a host of other, mostly neurological, problems (Blau et al 2010). Originally described by Asbjørn Følling in 1934 (Følling 1934), phenylketonuria was both the first identified metabolic disease as well as the first that could be treated. First implemented in the early 1950s, a low-phenylalanine diet is still the standard treatment of the disease (Macleod and Ney 2010). Only recently has this specialized diet been partially complemented by the administration of pharmacological doses of tetrahydrobiopterin (BH_4), the natural cofactor of deficient PAH (Levy et al 2007; Feillet et al 2008; Lee et al 2008; Trefz et al 2009). The observation of patients that responded with decreased plasma phenylalanine levels to the oral administration of sapropterin dihydrochloride (BH_4) (Kure et al 1999) prompted a study proving the pharmacological effect of BH_4 to correct hyperphenylalaninemia and restore PAH enzyme function in patients with milder phenotypes of phenylketonuria (Muntau et al 2002). The approval of sapropterin dihydrochloride as an orphan drug to treat BH_4 responsive PAH deficiency in 2007 (FDA) and 2008 (EMA) (Levy et al 2007; Feillet et al 2008; Lee et al 2008; Trefz et al 2009) marked a paradigm shift: using an oral pharmacological treatment to partially or even fully replace a working but arduous diet. Nowadays, approximately half of all phenylketonuria patients are assumed to benefit from this therapy. Among these patients, especially those with a mild to moderate clinical phenotype resulting in some residual enzyme activity, have the highest likelihood to respond to the cofactor treatment (Bernegger and Blau 2002; Muntau et al 2002; Hennermann et al 2005; Wang et al 2007; Zürfluh et al 2008; Dobrowolski et al 2011).

At the time of approval of sapropterin dihydrochloride to treat PAH deficiency, the mode of action of the new drug was not well understood. Considering missense mutations in the *PAH* gene lead to misfolding and loss of function of the PAH protein (Fig. 2a) (Pey et al 2003; Erlandsen et al 2004; Gersting et al 2008), the possibility that BH_4 exerted its effect as a pharmacological chaperone was examined. BH_4 responsiveness was disclosed in Pah^{enu1}, a mouse model for PAH deficiency. Loss of function resulted from loss of PAH, a consequence of misfolding, aggregation, and accelerated degradation of the enzyme. BH_4 attenuated this triad by conformational stabilization augmenting the effective PAH concentration and this led to the rescue of the biochemical phenotype and enzyme function in vivo. Combined in vitro and in vivo analyses revealed a selective pharmaceutical action of BH_4 confined to the pathological metabolic state (Gersting et al 2010).

Further work focused on the identification of BH_4 derivatives or new molecules that also stabilize the PAH protein but show more favorable pharmaceutical properties concerning synthesis, bioavailability, and stability. A high-throughput ligand screen of over 1000 pharmacological agents revealed two compounds that stabilized the functional tetrameric conformation of the PAH protein (Pey et al 2008). An integrated drug discovery approach with shape-focused virtual screening of the National Cancer Institute's chemical library identified 84 candidate molecules with potential to bind to the active site of PAH. Two compounds, benzylhydantoin and 6-amino-5-(benzylamino)-uracil, stabilized the PAH protein, substantially increased in vivo phenylalanine oxidation and by this reduced blood phenylalanine concentrations in Pah^{enu1} mice (Santos-Sierra et al 2012).

Tafamidis for the treatment of transthyretin-related hereditary amyloidosis

Transthyretin-related hereditary amyloidosis, also called familial amyloid polyneuropathy (ATTR, MIM 105210), is an autosomal dominant neurodegenerative disease with fatal outcome. It is characterized by progressive neuropathy and/or cardiomyopathy resulting from mutations in the *TTR* gene. ATTR is caused by aggregation of transthyretin (TTR), a natively tetrameric protein involved in the transport of thyroxine and the vitamin A-retinol-binding protein complex. Mutations within the *TTR* gene facilitate tetramer dissociation, monomer misfolding and aggregation. Because tetramer dissociation is the rate-limiting step in TTR amyloidogenesis, targeted therapies have focused on small molecules that

kinetically stabilize the tetramer, inhibiting TTR amyloid fibril formation (Hammarstrom et al 2003). One of these compounds, tafamidis meglumine (Fx-1006A), has received market approval. It binds selectively to the two normally unoccupied thyroxine-binding sites of the tetramer, and kinetically stabilizes TTR. The crystal structure of tafamidis-bound TTR suggested that binding stabilizes the weaker dimer-dimer interface against dissociation, the rate-limiting step of amyloidogenesis. By this mechanism, tafamidis slowed down disease progression in patients heterozygous for the *V30M TTR* mutation. Moreover, other patient-derived amyloidogenic variants of TTR, including kinetically and thermodynamically less stable mutants, were also stabilized by tafamidis binding suggesting its broader efficacy for ATTR (Bulawa et al 2012).

Compounds in preclinical or clinical stages of investigation

Pharmacological chaperone compounds for the treatment of cystic fibrosis

Cystic fibrosis (CF, MIM 219700) is a genetic disorder resulting from loss of chloride transport through the cystic fibrosis transmembrane conductance regulator (CFTR). Combined effort of clinical and basic science research has constantly increased the patient's life expectancy from a regularly fatal outcome in adolescence to more than 50 years for individuals born today. However, CF still is a burdensome disease associated with a need for lifelong treatment. The CFTR protein contains two membrane-spanning domains (MSD1-2), two nucleotide-binding domains (NBD1-2), and a regulatory domain. The most common *CFTR* mutation, Δ*F508* in NBD1, impairs domain folding, plasma membrane expression, function and stability, and induces premature degradation of the variant protein (Riordan 2008). The compound VX-809, identified in a high-throughput screening approach, is an investigational CFTR corrector that partially restores CFTR function in homozygous ΔF508 patients. It was shown to stabilize an N-terminal domain in CFTR that contains only MSD1 and to restore function to CFTR forms that have missense mutations in MSD1 (Okiyoneda et al 2013; Ren et al 2013).

A pharmacological chaperone compound for the treatment of methylmalonic aciduria (MMA) cblB type

The autosomal recessive disease methylmalonic aciduria, cblB type (MMA, MIM 251110), leads to encephalopathies of variable severity. This results from the genetic defect affecting the synthesis of the adenosylcobalamin cofactor for the enzyme methylmalonyl-CoA mutase. Following the description of mutations within the *MMAB* gene that are connected with cob(I)alamin transferase's (ATR) residual enzyme activity (Jorge-Finnigan et al 2010), a recent study intended to identify pharmacological chaperones for the treatment of MMA, cblB type (Jorge-Finnigan et al 2013). In a high-throughput ligand screening of more than 2000 compounds, several small molecules with effect on the thermal stability of ATR have been identified. One such compound, N-{[(4-chlorophenyl)carbamothioyl]amino}-2-phenylacetamide, was observed to significantly increase thermal stability of the purified protein while not working as an inhibitor. Additionally, it was observed to enhance the ATR activity in patient fibroblasts with a destabilizing hemizygous *I96T* mutation. Cobalamin, when present, increased this effect. An increase in steady-state levels of the ATR protein could be observed in both liver and brain of C57BL/6 J mice after a 12 day long oral administration of the compound in low dosages (Jorge-Finnigan et al 2013).

Pharmacological chaperone compounds for the treatment of primary hyperoxaluria

Primary hyperoxaluria type 1 (PH1, MIM 259900) is a disorder of glyoxylate metabolism. The defect is characterized by deficiency of the hepatic peroxisomal enzyme alanine: glyoxylate aminotransferase (AGT) leading to accumulation of oxalate. The illness causes a variable range of symptoms from occasional symptomatic kidney stones to deposition of calcium salts in the kidneys and renal arteries up to end-stage chronic kidney disease with systemic involvement. Missense mutations in the *AGT* gene affect the conformation of the enzyme (Fig. 2b). The changes in the enzyme's conformation also include oligomerization defects that induce a decrease in protein stability (Cellini et al 2010). This can lead to mistargeting of the peroxisomal protein toward mitochondria (Fargue et al 2013a). Response to supplementation with the pyridoxal phosphate cofactor proved effective in 10 to 30 % of all patients (Cellini et al 2011). In addition to the positive impact of pyridoxal phosphate on AGT enzyme kinetics, it is increasingly acknowledged to act as a pharmacological chaperone (Fargue et al 2013b). This is mechanistically supported by the observation of increased kinetic stability of AGT bound to the cofactor (Pey et al 2011).

Pharmacological chaperone compounds for the treatment of lysosomal diseases

Gaucher disease (GD, MIM 230800) denotes a progressive lysosomal storage disorder resulting from reduced glucosylceramidase (GCase) activity. This leads to the accumulation of the glycosphingolipid glucosylceramide in the lysosome and dysfunction in multiple organs. Several compounds have already been identified as having a corrective potential toward the misfolding phenotype in Gaucher disease (Fig. 2c). Isofagomine tartrate (Chang et al 2006; Steet et al

2006; Kornhaber et al 2008; Khanna et al 2010; Sun et al 2011, 2012) and the second generation compound AT3375 (Amicus Therapeutics 2013) have reached clinical stages in drug development and were shown to bear pharmacological chaperone activity by increasing GCase activity in patient cells. However, a combined approach of enzyme replacement therapy and administration of a pharmacological chaperone may be the most promising treatment strategy for this disorder (Amicus Therapeutics 2012).

Pompe disease (PD, MIM 232300) is a metabolic myopathy resulting from deficiency of the lysosomal enzyme α-glucosidase (GAA). Currently only enzyme replacement therapy with recombinant human GAA (rhGAA) has received approval. This approach, however, has shown shortcomings in regard to its therapeutic efficacy in a number of patients afflicted with Pompe disease and an alternative therapeutic strategy using pharmacological chaperones has been proposed. Yet, the chaperones identified were all active site-directed molecules and potential inhibitors of the targeted enzyme. The best pharmacological chaperone would be a molecule with high affinity for an allosteric site in the target protein, providing stabilization and at the same time evading inhibition of the protein's activity. Recently, the compound N-acetylcysteine (NAC) was shown to work as a novel allosteric chaperone for the GAA enzyme. This compound increased the residual activity of mutated GAA both in cultured fibroblasts obtained from patients and in COS7 cells overexpressing mutated GAA, while not disturbing the enzyme's catalytic activity (Porto et al 2012).

Fabry disease (FD, MIM 301500) is a genetic disorder due to deficiency of the lysosomal enzyme α-galactosidase A (α-Gal A) causing the accumulation of globotriaosylceramide (GL-3) in several tissues (Boustany 2013). Migalastat hydrochloride is an imino sugar shown to work as a pharmacological chaperone in Fabry disease. It selectively binds and stabilizes α-Gal A leading to enhanced cellular levels and activity for all those mutated variants of the enzyme with remaining residual activity (Fan et al 1999; Wu et al 2011; Giugliani et al 2013). A series of studies, both preclinical and clinical, on the application of migalastat hydrochloride were performed in cell culture, in Fabry transgenic mice (Young-Gqamana et al 2013), and in patients (Germain et al 2012; Giugliani et al 2013). The studies pointed toward an elevated α-galactosidase A activity in blood, skin, and kidney as well as a reduction in elevated concentrations of GL-3 in plasma, urine, and tissues with good tolerability when applying migalastat hydrochloride. However, the pivotal phase 3 placebo-controlled double-blind study failed to demonstrate the benefit of migalastat hydrochloride over placebo with statistical significance (Amicus Therapeutics 2012).

Krabbe disease or globoid cell leukodystrophy (GLD, MIM 245200) is an autosomal recessive, degenerative, lysosomal storage disease resulting from a loss of enzyme activity of galactocerebrosidase (GALC). Besides inducing a significant reduction in GALC activity, mutations in the *GALC* gene also cause a lack of correct protein processing into an N-terminal GALC fragment resulting in a considerable reduced amount of GALC localized to the lysosome. Both protein misfolding and hyperglycosylation can be attributed to the *D528N GALC* mutation. The use of α-lobeline, which functions as an inhibitor of GALC, was shown to significantly raise the activity of this variant GALC, pointing to a pharmacological chaperone role for the compound (Lee et al 2010b).

The innovative treatment approach of a small molecule based pharmacological chaperone therapy was first described for Fabry disease and a fundamental body of research has been generated since then in the field of lysosomal storage diseases. However, there is still no drug on the market for one of these indications. This underlines the challenge faced in the design of clinical trials for complex inborn errors of metabolism and the necessity for clinical endpoints to ideally assess protein function in vivo. The use of breath tests with the stable carbon isotope ^{13}C offers a safe and non-invasive way to monitor in vivo activity of an enzyme as has been demonstrated for a number of disorders (Muntau et al 2002; Yan et al 2006; Wu et al 2010).

Synergy of enzyme replacement therapy and pharmacological chaperones for the treatment of lysosomal storage disorders

The mainstay of treatment for lysosomal storage disorders is enzyme replacement therapy (Ratko et al 2013). However, not only enzymes affected by mutations, but also recombinant wild-type enzymes used for enzyme replacement therapy may display low stability and may thus be prone to mistrafficking and degradation. On the other hand, monotherapy approaches using pharmacological chaperones to rescue enzyme function in lysosomal storage diseases did not straightly prove efficient. Therefore, recent studies investigated the effect of a combined application of recombinant enzymes with a pharmacological chaperone in Fabry disease (Benjamin et al 2012) and in Pompe disease (Scavelli et al 2005; Porto et al 2012) and demonstrated that this approach leads to an increase in enzyme activity and a decrease in substrate accumulation. This demonstrates how pharmacological chaperones can protect recombinant enzymes from degradation and by this may improve and prolong the effect of well-established enzyme replacement strategies (Parenti et al 2013).

Proteostasis regulators

The therapeutic application of proteostasis regulators was first carried out in lysosomal storage diseases. Some misfolded variant forms of glucosylceramidase causing Gaucher disease did not respond to pharmacological chaperone treatment due to their rapid degradation by the endoplasmic reticulum-

associated degradation (ERAD). Celastrol and MG-132, a known heat shock transcription factor 1 (HSF1) activator and a proteasome inhibitor, enhanced folding, trafficking, and activity of these misfolded, non-pharmacological responsive glucosylceramidase variants (Mu et al 2008). Rescue of glucosylceramidase variants was also achieved by modulation of other proteostasis systems, like the Ca^{2+} signaling pathway. Lacidipine is an L-type Ca^{2+} channel blocker that increases folding, trafficking, and lysosomal activity of severe Gaucher disease variants in patient fibroblasts (Wang and Segatori 2013). Interestingly, the proteostasis regulators used were also effective in the treatment of other lysosomal storage diseases, pointing to their action on a proteostasis network rather than directly on the protein. Moreover, a synergistic effect for the combined application of proteostasis regulators and pharmacological chaperones was demonstrated (Mu et al 2008). This strategy may be beneficial for the treatment of more severe phenotypes, where the proteostasis regulator confers a corrective environment to the pharmacological chaperone to execute its action.

Cystic fibrosis is another inborn error of metabolism where the beneficial use of proteostasis regulators has already been demonstrated. A large HTS screen for proteostasis regulators has identified small molecules able to activate the HSR and the UPR responses and promoting rescue of the most common form of the disease, ΔF508 (Calamini et al 2012). The modulation of Ca^{2+} signaling pathways by inhibitors of the Ca^{2+}-ATPase SERCA in the endoplasmic reticulum, thapsigargin (Egan et al 2002), and curcumin (Egan et al 2004), resulted in escape of the variant protein to proteasomal degradation by the ERAD and enhanced trafficking and activity of ΔF508 CFTR. The use of histone deacetylases inhibitors (HDACi) such as SAHA induced improved trafficking and activity of the ΔF508 variant protein. The beneficial aspect in this case resulted from the inhibition of HDAC7 that led to inhibition of Hsp90 activity by acetylation, which in turn activated other proteostasis genes involved in CFTR folding (Hutt et al 2010). Compounds that promote downregulation of AHA1, a cochaperone of Hsp90, also improve folding and activity of variant CFTR (Wang et al 2006). Successful restoration of CFTR function by dissimilar proteostasis regulators acting on different proteostasis networks reveals the huge potential of correcting misfolding disorders by acting on the proteostasis network (Hutt and Balch 2013; Rowe and Verkman 2013; Sampson et al 2013).

Outlook

Protein misfolding with loss-of-function is a general phenomenon associated with missense mutations in human genetic disease. In the last years, this molecular phenotype has been uncovered in a large number of inborn errors of metabolism. The understanding of the underlying pathophysiology with distinct mechanisms in different compartments of the cell paved the way for the development of small molecule therapies. These aim to correct protein misfolding and to stabilize and increase the effective intracellular concentration of the protein affected by a mutation. Both, pharmacological chaperones and proteostasis regulators, have proven effective in restoring mutant enzyme homeostasis and can be used either alone or in combination to exploit synergistic effects.

Candidate molecules for pharmacological chaperones can be identified by two different strategies, hypothesis-free high-throughput screenings (HTS) exploring libraries of thousands of existing chemicals and drugs, or hypothesis-driven approaches. HTS are particularly valuable to identify novel and often unexpected chemical structures (Pey et al 2008) and are mainly directed to endoplasmic reticulum-associated pathologies (cystic fibrosis and lysosomal storage disorders), where stabilization of the variant proteins facilitates escape from degradation by the protein quality control system (Powers et al 2009). Notably, several compounds such as pharmacological chaperones for the treatment of Fabry disease and Pompe disease have already reached late stages of clinical trials (Valenzano et al 2011).

Hypothesis-driven strategies are usually based on screening for small molecular weight compounds that are structurally related to key moieties of the natural substrates, cofactors or inhibitors (Santos-Sierra et al 2012). Importantly, an extensive range of in vitro and in vivo assays are now available to evaluate physical interaction of these compounds with the variant proteins as well as to assess conformational stabilization, cellular levels and residual function of the affected proteins. Screens of compound libraries have been performed to identify hit molecules by monitoring thermal protein stability using differential scanning fluorimetry (Niesen et al 2007). Cell-based HTS assays have been widely used to show that hit compounds can enhance total cellular levels of a variant protein and rescue its biological function (Tropak and Mahuran 2007).

All these experimental strategies are increasingly exploited for the discovery of new small molecule compounds that belong to a still young class of pharmaceutical products, the class of pharmacological chaperones and proteostasis regulators. These compounds may in the near future improve existing treatment strategies or even offer a first-time treatment to patients suffering from nowadays-untreatable inborn errors of metabolism.

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Competing interest Ania C. Muntau is a member of a Scientific Advisory Board and a consultant to Merck Serono KGaA. João Leandro, Michael Staudigl, Felix Mayer, and Søren W. Gersting declare that they have no conflict of interest.

Appendix

Table 1 Protein misfolding in inborn errors of metabolism and small molecules acting as pharmacological chaperones or proteostasis regulators

Disease	OMIM	Incidence	Protein	Pharmacological chaperone/proteostasis regulator[a]	References
Cystic fibrosis	219700	1 : 2500	Cystic fibrosis transmembrane conductance regulator	2-(2-Benzoxazolyl)-3-(3-pyridinyl)-1-(3,4,5-trimethoxyphenyl)-2-Propen-1-one (*PR*); 4-Chloro-3-nitro-1-(phenylmethyl)-2(1H)-Quinolinone (*PR*); 5-[3-(4-Methoxyphenyl)-2-propen-1-ylidene]-2,4,6(1H,3H,5H)-Pyrimidinetrione (*PR*)	(Calamini et al 2012)
				4-phenylbutryate	(Rowe and Verkman 2013)
				Benzoquinolizinium; UCcf-029; UCcf-180	(Galietta et al 2001)
				CoPo-22	(Phuan et al 2011; Clancy et al 2012)
				Corr-4a	(Pedemonte et al 2005; Loo et al 2008)
				Curcumin (*PR*)	(Egan et al 2004)
				Glafenine (*PR*)	(Robert et al 2010)
				Phenylhydrazone RDR1	(Sampson et al 2011)
				SAHA (*PR*)	(Hutt et al 2010)
				siRNA Aha1 (*PR*)	(Wang et al 2006)
				Thapsigargin (*PR*)	(Egan et al 2002)
				VX-809	(Okiyoneda et al 2013; Ren et al 2013)
Fabry disease	301500	1 : 40,000	α-galactosidase A	Migalastat hydrochloride	(Fan et al 1999; Wu et al 2011; Germain et al 2012; Giugliani et al 2013; Young-Gqamana et al 2013)
Familial hypercholesterolemia	143890	1 : 500	Low-density lipoprotein receptor	4-phenylbutyrate	(Tveten et al 2007)
Familial hypocalciuric hypercalcemia type 1	145980	n.a.	Extracellular calcium-sensing receptor	NPS R-568	(Fox et al 1999)
Galactosemia	230400	1 : 40,000	Galactose-1-phosphate uridyltransferase	Arginine	(Vicente et al 2011)
Gaucher disease type I	230800	1 : 40,000 1 : 1,000 (Turkey)	β-glucocerebrosidase	Ambroxol	(Maegawa et al 2009; Bendikov-Bar et al 2011)
				AT3375	(Amicus Therapeutics 2013)
				Celastrol (*PR*); MG-132 (*PR*)	(Mu et al 2008)
				Isofagomine tartrate	(Chang et al 2006; Steet et al 2006; Kornhaber et al 2008; Khanna et al 2010; Sun et al 2011; Sun et al 2012)

Table 1 (continued)

Disease	OMIM	Incidence	Protein	Pharmacological chaperone/proteostasis regulator[a]	References
				Lacidipine (*PR*)	(Wang et al 2011; Wang and Segatori 2013)
				Miglustat (NB-DNJ)	(Alfonso et al 2005)
				N-(n-nonyl)deoxynojirimycin (NN-DNJ)	(Sawkar et al 2002)
				Sucrose (TFEB activator) (*PR*)	(Song et al 2013)
Glutaric aciduria type 1	231670	1 : 50,000	Glutaryl-CoA-dehydrogenase (GCDH)	FAD	(Lucas et al 2011)
G(M1)-gangliosidosis	230500	1 : 100,000	Lysosomal β-galactosidase	N-octyl-4-epi-beta-valienamine	(Suzuki et al 2007)
				Galactose	(Caciotti et al 2009)
G(M2)-gangliosidosis	272750 (AB variant) 268800 (Sandhoff) 272800 (Tay-Sachs)	1–9 : 1,000,000 (Sandhoff) 1 : 320 000 (Tay-Sachs)	β-hexosaminidase A	Bisnaphthalimide, nitro-indan-1-one, pyrrolo[3,4-d]pyridazin-1-one	(Tropak et al 2007)
				N-acetylglucosamine thiazoline (NGT)	(Tropak et al 2004)
				Pyrimethamine	(Clarke et al 2011)
				Sucrose (TFEB activator) (*PR*)	(Song et al 2013)
Hemophilia A due to factor VIII deficiency	306700	1 : 6000 males	Coagulation factor VIII	Betaine	(Roth et al 2012)
Hereditary hemochromatosis	235200	1 : 200 US Caucasian	Hereditary hemochromatosis protein (HFE)	4-phenylbutyrate, TUDCA	(de Almeida et al 2007; de Almeida and de Sousa 2008)
Homocystinuria	236200	1 : 20,000	Cystathionine beta synthase	Betaine, taurine	(Kopecká et al 2011)
				Bortezomid (*PR*), ONX 0912 (*PR*)	(Gupta et al 2013)
				DMSO, glycerol, proline, TMAO	(Singh et al 2007; Majtan et al 2010)
				Pyridoxine	(Chen et al 2006; Smith et al 2012)
				S-adenosyl-methionine	(Pey et al 2013)
Hyperinsulinemic hypoglycemia	256450	1 : 50,000	Sulfonylurea receptor-1 (ABCC8)	Diazoxide	(Flanagan et al 2010)
Krabbe disease	245200	1 : 100,000−1 : 250,000	galactocerebrosidase	α-lobeline	(Lee et al 2010b)
Maple syrup urine disease	248600	1 : 200,000	BCKD complex	TMAO	(Song and Chuang 2001)
Medium-chain acyl-CoA dehydrogenase deficiency	201450	1 : 8000	Medium-chain acyl-CoA dehydrogenase	FAD	(Lucas et al 2011)
Menkes disease	309400	1 : 50,000	Copper-transporting ATPase 1 (MNK Menkes protein)	Copper	(Kim et al 2002)
Methylmalonic aciduria, cblB type	251110	n.a.	Cob(I)alamin transferase	N-{[(4-chlorophenyl)carbamothioyl] amino}-2-phenylacetamide	(Jorge-Finnigan et al 2013)
Methylmalonic aciduria and homocystinuria, cblC type	277400	n.a.	Methylmalonic aciduria and homocystinuria type C protein	Vitamin B12	(Froese et al 2009)
Obesity due to melanocortin 4 receptor deficiency	601665	1–2 : 5000	Melanocortin-4 receptor (MC4R)	DCPMP, MPCI, MTHP, NBP, PPPone	(René et al 2010)

Table 1 (continued)

Disease	OMIM	Incidence	Protein	Pharmacological chaperone/proteostasis regulator[a]	References
Phenylketonuria	261600	1 : 6500	Phenylalanine hydroxylase	3-amino-2-benzyl-7-nitro-4-(2-quinolyl)-1,2-dihydroisoquinolin-1-one); 5,6-dimethyl-3-(4-methyl-2-pyridinyl)-2-thioxo-2,3-dihydrothieno[2,3- d] pyrimidin-4(1H)-one	(Pey et al 2008)
				6-amino-5-(benzylamino)-uracil; benzylhydantoin	(Santos-Sierra et al 2012)
				Sapropterin dihydrochloride (Kuvan®), tetrahydrobiopterin,	(Levy et al 2007; Gersting et al 2010)
Pompe disease	232300	1:40,000	Alpha-1,4-glucosidase	1-deoxynojirimycin (DNJ)	(Khanna et al 2012)
				N-acetylcysteine	(Porto et al 2012)
Primary hyperoxaluria type 1	259900	1 : 120,000	L-alanine-glyoxylate aminotransferase	Pyridoxine/pyridoxal phosphate	(Cellini et al 2011; Pey et al 2011; Fargue et al 2013b)
Progressive familial intrahepatic cholestasis type 1 [PFIC1]	211600	1 : 50,000−1 : 100,000	ATP8B1 (putative aminophospholipid-translocating P-type adenosine triphosphatase)	4-phenylbutyrate	(van der Velden et al 2010)
Pyruvate carboxylase deficiency	266150	1–9 : 1,000,000	Pyruvate carboxylase	Biotin	(Feldman et al 1981)
Pyruvate dehydrogenase E1-alpha deficiency	312170	n.a.	Pyruvate dehydrogenase complex, subunit E1-alpha	Arginine	(João Silva et al 2009)
Short chain acyl-CoA dehydrogenase deficiency	201470	1 : 40,000−1 : 100,000	Short-chain acyl-CoA dehydrogenase	FAD	(Lucas et al 2011)
Transthyretin-related hereditary amyloidosis (familial amyloid polyneuropathy)	105210	1–9 : 1,000,000	Transthyretin	Tafamidis (Vyndaqel®)	(Bulawa et al 2012)

[a] The proteostasis regulators are indicated by (*PR*) after the compound's name. n.a., not available

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