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Review

Proteostasis in thermogenesis and obesity

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Abstract

The proper production, degradation, folding and activity of proteins, proteostasis, is essential for any cellular function. From single cell organisms to humans, selective pressures have led to the evolution of adaptive programs that ensure proteins are properly produced and disposed of when necessary. Environmental factors such as temperature, nutrient availability, pathogens as well as predators have greatly influenced the development of mechanisms such the unfolded protein response, endoplasmic reticulum-associated protein degradation and autophagy, working together in concert to secure cellular proteostasis. In our modern society, the metabolic systems of the human body face the distinct challenge of changed diets, chronic overnutrition and sedentary lifestyles. Obesity and excess white adipose tissue accumulation are linked to a cluster of metabolic diseases and disturbed proteostasis is a common feature. Conversely, processes that promote energy expenditure such as exercise, shivering as well as non-shivering thermogenesis by brown adipose tissue (BAT) and beige adipocytes counteract metabolic dysfunction. Here we review basic concepts of proteostasis in obesity-linked metabolic diseases and focus on adipocytes, which are critical regulators of mammalian energy metabolism.

Keywords: adipocyte, endoplasmic reticulum, obesity, proteostasis, thermogenesis.

Introduction: the global crisis of obesity and related metabolic diseases

Nutrients impact the development, growth, and reproduction of mammals. To secure cellular and organismal homeostasis and thereby thrive amidst variations in nutrient availability, an array of molecular mechanisms that coordinate metabolic adaptations to nutrient fluctuations have been acquired through evolution. Nowadays, humans in modern societies face a distinctly new challenge, as the year-round exposure to conveniently available energy dense foods has resulted in a worldwide rise in chronic overnutrition and, as a result, an enormous increase in the prevalence of obesity which frequently corresponds with the accumulation of excess and dysfunctional adipose tissue. In fact as of 2015 the prevalence of obesity exceeded 600 million for adults and 107 million for children (Afshin *et al.*, 2017). Given the strong link between obesity and metabolic diseases such as type 2 diabetes, stroke, heart failure, and liver disease, as well as certain cancers and neurodegenerative disorders, obesity has become a grave medical and socioeconomic concern. Correspondingly, there is now a growing scientific and medical interest in understanding the molecular basis of metabolic adaptations and how these programs are altered in human obesity.

Obesity develops when energy intake exceeds energy expenditure, for example in people consuming energy-dense meals and when physical activity is low. Major advances have been made in defining the neuronal and hormonal circuitry that regulate satiety and energy expenditure, and how dysregulation of these processes contribute to adiposity. We have gained deep molecular insight from rare cases of monogenetic human obesity and the corresponding naturally occurring obese rodent strains. In these cases, signaling of leptin, a fat cell-derived hormone with a multitude of effects on energy metabolism is abrogated (Farooqi and O'Rahilly, 2006). Loss-of-function mutations in the gene coding for Leptin or the Leptin receptor are most prominently causally related to diminished satiety and a complex aberrant metabolism. In general, being able to metabolize excess calories to lipids that are stored in specialized fat cells, the adipocytes, for times when nutrient supply is scarce is likely an evolutionary advantage. However, in chronic states of obesity the storage capacity of adipocytes will eventually be unable to comply with the demand, leading to ectopic lipid accumulation in tissues that have far less capacity to adapt to the metabolic burden that stored lipids and other types of nutrients impose on cellular processes (Oikonomou and Antoniades, 2019). The disruption of lipid homeostasis is also linked to aberrant protein and carbohydrate metabolism, which altogether causes stress and inflammatory responses. This hallmark of

chronic obesity, a low-grade inflammatory response of the innate and adaptive immune system, is evident in most tissues that are critical for systemic metabolic homeostasis, such as adipose, skeletal muscle, pancreas, and liver (Hotamisligil, 2017; Lee *et al.*, 2018) (Figure 1). Ultimately, failed metabolic adaptation in these tissues causes chronic insulin resistance and insulin insufficiency, which thereby drives abnormal glucose and lipid metabolism, and ultimately the development of diabetes and cardiovascular disease (Oikonomou and Antoniades, 2019).

In contrast to the effects of excess nutrient storage, therapies that promote energy expenditure bestow a multitude of beneficial effects on metabolic health in people with obesity, for example increased physical activity and exercise (Villareal *et al.*, 2011). Likewise, thermogenesis is a physiological process that has a profound impact on energy metabolism. All biochemical processes lead to loss of reaction energy in the form of heat, and the portfolio of enzymes has developed towards being efficient to limit this unwanted dissipation of chemical energy. However, reaction temperature is a critical factor in biochemical reactions and therefore heat generation and body temperature are key elements of animal physiology. The heat generated during endogenous biochemical processes is an important contributor to the basic metabolic rate and homeothermy in mammals (Jastroch *et al.*, 2018). An important point in evolution was when animals were no longer reliant on solar heat to increase their body temperature. Instead, below certain environmental temperatures there is a rise in metabolic rates that increases heat production and this component of homeothermy is called adaptive thermogenesis. In response to cold exposure, shivering thermogenesis in muscle, as well as non-shivering thermogenesis in thermogenic adipocytes is activated (Bartelt and Heeren, 2014). While the first phase of cold acclimatization is mediated by shivering thermogenesis it is progressively replaced by non-shivering thermogenesis (Cannon and Nedergaard, 2004). Thermogenic adipocytes share most of the features of classical white adipocytes. In addition, they display high mitochondrial content, high rates of oxygen consumption and are best distinguished by the expression of the uncoupling protein-1 (UCP1), a protein that drives heat production by creating a proton leak across the inner mitochondrial membrane. In humans, they can be found in depots referred to as classical brown adipocytes or dispersed in white adipose tissue as beige adipocytes, and these cells can convert large amounts of nutrients into heat in order to maintain body temperature. The activation of thermogenic adipocytes promotes numerous beneficial effects on metabolic health in humans and in preclinical mouse models of obesity, diabetes and atherosclerosis

(Bartelt *et al.*, 2011, 2017a; Berbee *et al.*, 2015). However, the contribution of non-shivering thermogenesis to human energy expenditure is negligible in most people unless their brown fat is "trained" by cold exposure. In addition, the activity of thermogenic adipocytes has been found to be significantly reduced in human obesity and with ageing (Cypess *et al.*, 2009; Saito *et al.*, 2009), which altogether is currently a major limitation for the potential impact of this type of therapeutic intervention. Thus, an important question in the field is how the metabolic activity of thermogenic cells can be sustained amidst high nutrient flux and how this can be exploited as a therapy for metabolic disease. In this review, we discuss 3 aspects related to these questions. First, we will discuss key features of metabolic stress in obesity and how it relates to metabolic disease. Second, we will examine a major consequence of metabolic stress, defective proteostasis. Third, we will discuss the high metabolic capacity of thermogenic cells and how this predisposes these cells to defective proteostasis. The highly specialized constitution of thermogenic adipocytes requires specialized mechanisms to adapt, and we will discuss the discovery of a specialized mechanism by which thermogenic cells sustain proteostasis via a transcriptional feedback pathway. We will conclude by discussing the potential relevance of this pathway to metabolic health & disease in obesity.

Role of metabolic stress in obesity-linked metabolic diseases

Balanced nutrient metabolism, occurring in regular fasting and feeding cycles, is managed by physiologic adaptive molecular programs in cells that are key to regulating systemic metabolic homeostasis such as adipocytes, hepatocytes, immune cells, pancreatic beta cells and specific neurons in the hypothalamus. At the core of metabolic adaptation is not only the regulation of metabolic reactions on a biochemical level, but also the coordinated adaptation of cellular organelles, such as the mitochondria and endoplasmic reticulum (ER), and other fundamental cellular processes, such as the homeostatic control of protein abundance, composition, and quality in relation to the metabolic demand, usually referred to as proteostasis. However, chronic exposure to high levels of nutrients elicits numerous types of stress, and, typically, elevated energy flux leads to excess storage of nutrients, ectopic accumulation of metabolic intermediates, or aberrantly modified cellular components (Hotamisligil, 2010).

Four important factors contribute to the timing and severity of metabolic dysregulation. First, the excess intake of calories, especially those rich in lipids and carbohydrates, drives the

weight gain that underlies obesity-induced metabolic dysfunction (Bartelt and Heeren, 2012). Second, the amount of meals and number of fasting-feeding cycles is an important factor in setting adaptive versus maladaptive metabolic programs (Ludwig *et al.*, 2019). Third, the biological rhythm and the inner clock is critical for priming the body for metabolic fluctuations (Panda, 2019). Lastly, ageing itself and the absolute time a person lives in the obese state is a strong risk factor for developing metabolic disease (Childs *et al.*, 2015).

Adaptive cellular responses engage multiple regulatory processes in an attempt to resolve the stress of excess nutrient intake. In obesity and associated disorders, the conserved programs of metabolic adaptation can fail, as the cells critical for systemic metabolism develop a progressive loss of the quality control mechanisms that govern healthy metabolism. If unresolved, this cellular stress becomes chronic and will eventually reach a threshold, after which the engagement of stress responses become maladaptive and toxic. Depending on the type of nutrient, this can lead to proteotoxic, lipotoxic or glucotoxic stress (Gregor and Hotamisligil, 2011). Regardless, a hallmark of the stress responses is the secretion of chemokines and cytokines by parenchymal cells that initiate immune cell recruitment and an inflammatory response in the tissue, which may represent an attempt to maintain or restore tissue function (Kotas and Medzhitov, 2015). However, in the setting of obesity the inflammation becomes chronic and ultimately, instead of resolving the stress, mediates disease progression (Hotamisligil, 2017; Lee *et al.*, 2018).

From our perspective, a promising strategy to treating obesity-linked chronic metabolic disease is to identify the molecular mechanisms by which key metabolic cells naturally adapt to the challenges or stress that result from high nutrient influx and developing ways to harness these processes to improve systemic metabolic homeostasis in the context of obesity (Figure 2). This strategy has already been proven effective, as the development of incretin hormone-based therapies arose out of the study of how this hormone system triggers systemic adaptations to nutrient ingestion (McIntosh *et al.*, 2010). This has turned out to be beneficial for the control of plasma glucose levels and emerged as an important class of anti-diabetic therapies (Campbell and Drucker, 2013; Mulvihill and Drucker, 2014). In addition to modulating this hormone system, targeting intrinsic stress resistance mechanisms in metabolically relevant cells might lead to potentially powerful therapeutics for a cluster of obesity-linked metabolic diseases.

Over the last two decades, great effort has been targeted at defining the underlying cause of metabolic diseases. Cellular stress in adipose, liver, and beta cells has been attributed as a major cause, although the source of stress, the manner in which stress is induced, and which type of stress has the greatest impact on metabolism remains a controversial topic. One converging point however is that these stresses are commonly linked to aberrant protein function and dysregulated protein turnover; in other words, there is a defect in proteostasis (Balchin *et al.*, 2016). For the remainder of this review, we will focus on the role of proteostasis in mammalian metabolism and the adaptive and maladaptive programs regulating proteostasis.

Proteostasis in metabolic adaptation and maladaptation

Proteostasis is a critical feature of any healthy cell. In order to sustain proteostasis, cells are equipped with three main programs, the unfolded protein response (UPR), the ubiquitin-proteasome system (UPS), and autophagy (Figure 3). In contrast, impairments in these processes cause protein aggregation, ER stress, and cellular dysfunction, which, if sustained, will usually have fatal consequences for the cell. During evolution, cells acquired these sophisticated programs to ensure that proteostasis is maintained under challenging environmental conditions (Ron and Walter, 2007). All biochemical reactions depend on temperature, hence plants and lower poikilotherm organisms are equipped with heat-shock or anti-freeze proteins to enhance protein folding and function during fluctuations in environmental temperature (Horowitz, 2014). In homeothermic animals, body temperature in core and peripheral areas is regulated and varies, depending on daytime, cold exposure, inflammatory status and more (Cannon and Nedergaard, 2004). In homeothermy, biochemical processes are largely independent of the environmental temperature and therefore heat-shock proteins might seem obsolete. However, heat-shock proteins are generally highly expressed in mammalian cells and regulated under physiological and pathological conditions. An important example of how nutrient metabolism is linked to proteostasis is the heat-shock 70 kDa protein 5, also known as GRP78 or BiP, encoded by the *Hspa5* gene. Hspa5 induction is commonly used as a surrogate marker of cellular proteotoxicity and ER stress, and Hspa5 levels are increased under several conditions of metabolic stress, for example in the livers of obese animals (Ozcan *et al.*, 2004). One main function of Hspa5 is to operate as an ATP-dependent protein chaperone that enhances protein folding, particularly during UPR

activation. Once protein folding is suboptimal, cells engage the UPR, which is classically considered to be triggered by three largely independent branches, inositol-requiring enzyme-1 (IRE1), Activating transcription factor-6 (ATF6) and protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), which are ER transmembrane proteins sensing luminal proteostasis (Ron and Walter, 2007). Hspa5 binds to each of the luminal domains of these proteins and when folding demand increases, Hspa5 is released from these transmembrane proteins to facilitate protein folding, and this activates the UPR sensors to engage countermeasures, collectively referred to as the UPR. This includes halting 5' cap dependent protein translation to reduce the protein synthesis load placed on the ER, and correspondingly inducing the transcription of chaperones that are alternatively synthesized via internal ribosomal entry sequence-dependent protein translation. If the ER stress remains unresolved for a prolonged period, the UPR will ultimately trigger apoptosis of the cell (Ron and Walter, 2007).

In obesity, the UPR has been shown to be activated in the liver, adipose, and beta cells (Hotamisligil, 2010). However, the mode of action of the UPR in the obese context has several distinctions from the classical paradigm that would be observed upon treating cells with ER stress-inducing compounds. For example, the source of obesity-induced ER stress in the liver involves alterations in ER membrane composition, calcium handling, and mitochondrial interactions (Arruda and Hotamisligil, 2015; Arruda *et al.*, 2014; Fu *et al.*, 2011, 2012; Widenmaier *et al.*, 2017), combined with defects in misfolded protein degradation via the proteasome (Otoda *et al.*, 2013) and via autophagy (Yang *et al.*, 2010). Moreover, inflammation in the obese liver alters IRE1 signaling to cause maladaptive UPR actions that worsens glycemic control (Yang *et al.*, 2015). However, it is important to be aware that the ER of hepatocytes is highly dynamic and unique in structure and abundance. Other cell types that play important roles in nutrient sensing and metabolism such as adipocytes, myocytes and neurons display distinct ER phenotypes. Hence, it is plausible that our relatively profound understanding of ER alterations in the liver in response to obesity might not relate to the pathophysiology in all of these cell types. Thus, a detailed cell type-specific understanding of ER biology, *in vivo*, is crucial for determining how to resolve the cellular proteotoxicities in metabolic tissues that underlie obesity-linked metabolic disease.

Complementary to the UPR, cells are also able to execute the degradation of unwanted, damaged or obsolete protein by the UPS, of which ER-associated protein degradation (ERAD) is important for co-translational protein quality control, and this interdependency

between UPR and UPS/ERAD has been highly conserved during eukaryotic evolution (Friedlander *et al.*, 2000; Travers *et al.*, 2000). The proteasome is a large macromolecular complex that is tasked with degrading proteins that have been targeted for destruction via the modification with ubiquitin (Collins and Goldberg, 2017). These proteins largely follow the N-degron or C-degron rule, meaning these proteins carry a degradation signal whose main determinants are, respectively, their N-terminal and C-terminal residues. A few examples exist where proteins are targeted for proteasomal destruction independently of ubiquitin (Erales and Coffino, 2014), such as the proteasomal subunit and regulator Rpn1, encoded by Psmd9 (Ju and Xie, 2004). While canonical N-degrons and C-degrons are not limited to targeting proteins for UPS and also lead to non-proteasomal proteolytic degradation, it is well established that especially short-lived proteins are destabilized by N-degrons, inducing their timely degradation by the proteasome (Varshavsky, 2019). In humans, mutations in the genes encoding for proteasome subunits (encoded by the Psm genes) are characterized by a disturbed immune system and aberrant metabolism (Brehm *et al.*, 2015; de Jesus *et al.*, 2019) and recent studies suggest an important role of the proteasome in hepatic lipid metabolism (Parker *et al.*, 2019). While cells can dynamically produce and disassemble proteasomes, as most proteasome subunits themselves are subject to UPS-mediated degradation (Bartelt *et al.*, 2018), it remains unclear under which conditions the proteasome is constitutively required and whether there are circumstances where proteasomal activity needs to be recruited in an adaptive fashion to meet varying demands. In addition, it remains unclear what the primary defect underlying compromised proteasome function is and to what extent phenotypic alterations are secondary to impaired proteostasis and cellular stress. Generally, the view has been that the proteasome is a static complex that is not under direct regulatory control. Instead that rates of protein degradation are dictated by tagging proteins with ubiquitin for degradation by the family of ubiquitin E3 ligases, for example under conditions of cancer cachexia (Bodine *et al.*, 2001). But new findings indicate that the biological reality is much more complex. In recent years many studies have revealed that the enzymatic activity of the proteasome can be directly regulated at the transcriptional (Bartelt *et al.*, 2018; Radhakrishnan *et al.*, 2010, 2014; Steffen *et al.*, 2010; Zhang *et al.*, 2014) and post-translational level (Rousseau and Bertolotti, 2016; VerPlank and Goldberg, 2017; VerPlank *et al.*, 2019; Zhao *et al.*, 2015). A key anabolic modulator called the mechanistic target of rapamycin complex-1 (mTORC1), on which multiple signaling mechanisms converge, e.g. mTORC1 is regulated by insulin, has emerged as an important modulator of these regulatory processes (Zhang *et al.*, 2014). Interestingly, the fasting-induced hormone glucagon has also been found to modulate

proteasome activity in hepatocytes in a cAMP-dependent manner (VerPlank *et al.*, 2019). Thus, it is possible that during physiological fasting and feeding states, insulin and glucagon and potentially other metabolic hormones dynamically modulate the proteasome to sustain proteostasis. Furthermore, it is tempting to speculate that under these conditions the proteostatic mechanisms such as UPR, ERAD and autophagy are integrated and regulated in a cell-type and context-dependent manner.

Proteasomal activity as an adaptive component of proteostatic quality control in thermogenic adipocytes

In light of the various phenotypes of mammalian cells, in terms of organelle composition, metabolic activities, and excess energy storage capacity, it is possible that mechanisms of metabolic adaptation might be different from cell to cell and dynamically regulated under various physiological conditions. In comparison to hepatocytes and beta cells (Eizirik *et al.*, 2008; Haataja *et al.*, 2008; Lee, 2014; Scheuner and Kaufman, 2008), much less is known about the mechanisms underlying proteostasis in adipocytes, particularly in the context of obesity. Adipocytes are structurally unique in that they are specialized to store fat. White adipocytes are made of 95% lipids and the content of lipids in thermogenic fat cells is also much greater than any other cell type that is involved in the maintenance of glucose and lipid metabolism (Bartelt *et al.*, 2017b). Adipocytes generally display relatively low amounts of ER and hardly any cytosolic volume. Therefore, the challenges during lipid droplet remodeling in obesity or, for example, during cold adaptation require specialized proteostatic mechanisms to match the make-up of adipocytes with the metabolic challenge. Several studies have addressed the role of ER stress in cultured white adipocyte models. In most cases, the experimental approach is using chemical compounds such as tunicamycin, which blocks N-linked glycosylation or thapsigargin, which blocks ER calcium uptake by inhibiting sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA). Rather than mimicking physiologic ER challenges, these molecules induce “proteocrisis”, as proteins are no longer folded properly and accumulate in the ER. In adipocytes, these compounds strongly induce UPR and unsurprisingly this results in impaired adipocyte differentiation in classical in vitro models. A major mechanism is the suppression of protein translation via PERK-mediated phosphorylation of eIF2alpha, which will have fate-altering or even fatal consequences for a differentiating cell (Han *et al.*, 2013). In mature white adipocytes, an important physiological

condition that involves UPR is lipolysis. During lipolysis, fatty acids are liberated from triglycerides in lipid droplets by adipose triglyceride lipase, abbreviated as ATGL (Zimmermann *et al.*, 2004). As fatty acids are amphiphilic molecules, they can accumulate in membranes, and if excessive, this can cause cellular stress. These disturbances are sensed by the ER-resident UPR machinery and dealt with via immediate reesterification of fatty acids into triglycerides by diacylglycerol transferase-1,abbreviated as DGAT1 (Chitraju *et al.*, 2017). In contrast to white adipocytes, the challenges thermogenic adipocytes face during cold adaptation are even more complex. Especially during non-shivering thermogenesis, which is a complex process that requires more than just higher Ucp1 activity and energy dissipation. Upon acute cold activation, thermogenic adipocytes utilize cellular glycogen and triglyceride stores to enhance respiration and Ucp1-mediated heat generation (Cannon and Nedergaard, 2004). However, this is limited by intracellular nutrient availability, so if the cold exposure is sustained the cells will need to replenish fuel by increasing uptake from the circulation (Bartelt *et al.*, 2011). Somewhat paradoxically, in parallel to these catabolic pathways, an additional feature of cold adaptation by thermogenic adipocytes is that it is anabolic in nature (Cannon and Nedergaard, 2004). This most prominently includes higher levels of de novo lipogenesis (Sanchez-Gurmaches *et al.*, 2018) as well as increased mitochondrial biogenesis (Wu *et al.*, 1999), which prepares the cell for sustained cold exposure, a process referred to as cold acclimatization. Not unexpectedly, mTORC1 plays an important role in this process and specific loss of mTORC1 results in severe BAT atrophy (Labbe *et al.*, 2016). The high synthetic demands in thermogenic adipocytes during cold adaptation generates stressful challenges that can disturb cellular proteostasis at the level of protein synthesis, protein folding and protein quality control. However, despite having multiple heavy demands on the ER synthesis capacity, the ER organelle in brown adipocytes is barely detectable via EM imaging (Bartelt *et al.*, 2018) and is dramatically less than in other metabolic cells such as hepatocytes and beta cells (Arruda *et al.*, 2014). It is conceivable that such low amounts of ER cause brown adipocytes to be hypersensitive to imbalances in proteostasis. Interestingly, UPR is activated upon cold exposure in brown adipocytes (Asada *et al.*, 2015) but, in sharp contrast to other cell types, genetic deletion studies suggest that the classical UPR branches are dispensable (Bartelt *et al.*, 2018; Gregor *et al.*, 2013). Neither conditional deletion of adipocyte IRE1 nor XBP1 impact on non-shivering thermogenesis in mice (Bartelt *et al.*, 2018). In contrast, there is some evidence that Xbp1 overexpression in adipocytes modulates plasma uridine levels, which indirectly stimulates energy balance through leptin signaling in the brain (Deng *et al.*, 2017). Also, other UPR components such as

PERK, ATF3, ATF4 and ATF6 seem to play no major role in thermogenic adipocytes (Bartelt, unpublished observations). Alternatively, brown adipocytes might engage autophagy as a proteostatic quality control process. However, there is little evidence that macroautophagy is physiologically regulated in non-shivering thermogenesis. Based on genetic deletion experiments, the autophagy components Autophagy-related 5 (ATG5) and ATG7 apparently operate as suppressors of non-shivering thermogenesis, since mice lacking adipocyte ATG5 (Kim *et al.*, 2019) or ATG7 (Singh *et al.*, 2009) display increased energy expenditure. A critical organelle for non-shivering thermogenesis are mitochondria and their function relies on proper fission and fusion to maintain a healthy mitochondria pool by eliminating damaged mitochondria through mitophagy (Liesa and Shirihai, 2013). Acute activation of mitochondrial respiration by cold is associated with increased fission (Wikstrom *et al.*, 2014). Mitochondrial fusion is also important for proper thermogenic function, as genetic deletion of Mitofusin-2 in adipocytes is associated with reduced thermogenic function and increased adiposity in mice (Boutant *et al.*, 2017; Mahdaviani *et al.*, 2017; Mancini *et al.*, 2019) and humans (Rocha *et al.*, 2017). Likewise, it seems that the Pink1-Parkin system is not required for the adaptation to cold but rather for disposing of obsolete mitochondria in brown adipocytes once non-shivering thermogenesis is no longer required to support the homeothermic needs of the host organism (Altshuler-Keylin *et al.*, 2016; Cairo *et al.*, 2019; Lu *et al.*, 2018).

So how do brown adipocytes adapt to the burdens of chronic cold exposure and obesity to secure proteostasis? The apparent lack of relevance for classical proteostatic, organelle-modulating mechanisms in thermogenic adipocytes led us to consider unconventional, alternative mechanisms of proteostasis during cold adaptation. As explained above, cells execute the UPR to fix problematic proteins. However, they can also dispose of troubled proteins by utilizing the UPS. Dynamic regulation of the UPS in physiology has rarely been observed and is usually associated with pathological conditions, most prominently neurodegenerative diseases and cancer cachexia (Goldberg, 2003). That protein degradation is an adaptive mechanism to support cell physiology has been generally underappreciated. Interestingly, it has been long known that Ucp1 undergoes relatively rapid protein turnover (Puigserver *et al.*, 1992) and is degraded by the UPS (Clarke *et al.*, 2012). This suggested to us that the levels of Ucp1 and potentially other proteins are regulated by the UPS during cold adaptation. Indeed, cold adaptation is associated with dynamically regulated global ubiquitin levels (Bartelt *et al.*, 2018). To date, the origin of the cold-induced increase in ubiquitin levels

remains unclear and the identity of the molecular drivers of deubiquitination and ubiquitination remain unknown. In fact, as cold is also associated with several-fold higher proteasomal activity, the levels observed are likely a dynamic newly adjusted steady state balance (Bartelt *et al.*, 2018). So, is this dynamic regulation an important mechanism for thermogenic adipocytes to adapt to cold? The answer to this question was made by taking advantage of small-molecule proteasome inhibitors such as bortezomib and carfilzomib that are an important clinical drug and are associated with a broad range of side effects. They can compromise ER function and induce pronounced ER stress (Bartelt *et al.*, 2018), likely caused by the accumulation of ubiquitinated proteins clogging the flow of protein turnover. We have tested whether the inhibition of cold-induced proteasomal activity by bortezomib or carfilzomib is meaningful and leads to temperature-related "side effects" in mice. Interestingly, at thermoneutrality, where thermogenic activity is minimal, certain effective doses of proteasome inhibitors do not impact whole-body energy expenditure, BAT function or body temperature. However, below 30 °C, when mice are exposed to cold, thermogenic adipose tissue displays ER stress and diminished thermogenesis when these doses of proteasome inhibitors are applied. These findings indicate that the increased thermogenic activity needs to be matched with corresponding increases in proteasome activity and proteostasis. At this point it is very difficult to conclude whether this is only a means to dispose of obsolete protein "trash" or whether ubiquitination in the brown fat proteome (Bartelt *et al.*, 2018) is also a regulatory modification.

How is this adaptive proteasomal activity regulated? An important adaptive modulator of the proteasome is the cap'n'collar transcription factor nuclear factor erythroid 2-related factor 1, Nfe2l1, also known as Nrf1 or Tcf11 (Kim *et al.*, 2016). Nfe2l1 is ubiquitously expressed and highly conserved in mammals (Kim *et al.*, 2016). Even though the Nfe2l1 gene was cloned decades ago (Chan *et al.*, 1993), there are still large gaps in our knowledge about this critical proteostatic factor. This is in part due to the fact that Nfe2l1 whole-body deletion is embryonic lethal (Chan *et al.*, 1998), and also because the biology of Nfe2l1 is complex and unique (Kim *et al.*, 2016). Nfe2l1 is an ER-transmembrane protein that undergoes post-translational modifications via glycosylation and ubiquitination, and there is still debate about the orientation of the protein and its ER-luminal-cytosolic translocation (Radhakrishnan *et al.*, 2014). In mammalian cells, its transcription factor-containing DNA-binding domain is proteolytically liberated from the ER transmembrane by the protease DNA damage inducible 1 homolog 2, abbreviated as DDI2 (Koizumi *et al.*, 2016). While multiple functions have been

attributed to Nfe2l1, many studies have highlighted its important role in protecting cells against insufficient proteasome activity in response to proteasome inhibitors (Radhakrishnan *et al.*, 2010; Steffen *et al.*, 2010). This function has been replicated in many scenarios, both *in vitro* (Radhakrishnan *et al.*, 2014; Sha and Goldberg, 2014; Zhang *et al.*, 2014) and *in vivo* (Bartelt *et al.*, 2018; Lee *et al.*, 2013). In a simplified model that only partially reflects all the experimental evidence, this cleaved active form of Nfe2l1 is itself degraded by the proteasome. This is a sophisticated feedback system to maintain proper proteasomal activity, as when proteasomal activity is insufficient to cope with the flow of ubiquitinated proteins, this cleaved "active" form of Nfe2l1 escapes degradation and, in turn, induces the transcription of all components necessary to increase the rate of protein degradation by the UPS (Sha and Goldberg, 2014) (Figure 4). It is important to point out that in most cell types Nfe2l1 is not essential for baseline proteasome levels. Instead, it appears that the adaptive recruitment of elevated proteasomal activity above "normal" cellular states is dependent on Nfe2l1. Interestingly, the cold-induced increase in proteasomal activity in thermogenic adipose tissue is paralleled by a transcriptional increase in Nfe2l1 (Bartelt *et al.*, 2018). It is unclear what drives cold-induced Nfe2l1 gene expression in thermogenic adipocytes. In liver, the transcription of the Nfe2l1 gene can be regulated by mTORC1 (Zhang *et al.*, 2014). In the absence of cold or thermogenic activity and even during the first hours of short-term cold exposure, mice lacking Nfe2l1 do not display a phenotype (Bartelt *et al.*, 2018). Rather, Nfe2l1 is required for the adaptation to long-term, sustained cold, which, as described above, is when these cells are under high catabolic and anabolic demands. Therefore, Nfe2l1 is a unique mediator of thermogenic adaptation and helps to establish a new metabolic set point. Nfe2l1 is regulated both in brown and beige adipocytes, and loss of Nfe2l1 in these cells induces a complex cellular response that only after days ultimately results in the apparent whitening of BAT and disappearance of beige cells from white adipose tissue (Bartelt *et al.*, 2018). It is important to point out that insufficient proteasomal activity in adipocytes results in signs of UPR activation, heat-shock response and autophagy, all of which are not able to restore proteostasis in the absence of Nfe2l1 (Bartelt *et al.*, 2018). The phenotype is progressive and exhibits prominent features of ER stress and aberrant mitochondrial function. The stress response induced by the adipocyte leads to the production of pro-inflammatory cytokines and chemokines that promote tissue inflammation and insulin resistance (Bartelt *et al.*, 2018).

Importantly, Nfe2l1 expression positively correlates with Ucp1 levels and thermogenic competency in human BAT (Bartelt *et al.*, 2018; Xue *et al.*, 2015). Hence, targeted regulation of Nfe2l1 may be clinically relevant for treating obesity-linked chronic metabolic disease. However, while the above studies highlight the proteostatic component of metabolic adaptation that is mediated by the Nfe2l1-pathway, they also raise several open questions for BAT research and beyond. For one, the general mechanism of upstream events requiring and leading to increased Nfe2l1 activity in thermogenic adipocytes is unclear. For another, even though the transcriptional induction of Nfe2l1 by cold is unique for BAT (Bartelt *et al.*, 2018), Nfe2l1 is ubiquitously expressed in the human body and so an obvious question relates to the role of Nfe2l1 in other metabolically active cells. Similar to our work in brown fat, Nfe2l1 was also recently implicated in white adipocytes (Hou *et al.*, 2018). But what Nfe2l1 does there and how this relates to human obesity remains unclear. In the liver, Nfe2l1 is regulated during fasting and feeding cycles (Zhang *et al.*, 2014) and its deletion causes ER stress and aberrant lipid metabolism (Lee *et al.*, 2013). Nfe2l1 activity is also linked to metabolite levels. We have recently shown that Nfe2l1 processing and activity is directly modulated by the levels of ER membrane cholesterol (Widenmaier *et al.*, 2017). Nfe2l1 can directly sense cholesterol by binding (Widenmaier *et al.*, 2017). When cholesterol levels in the ER rise, Nfe2l1 is retained and its decreased nuclear abundance promotes cholesterol detoxification pathways by transcriptional derepression (Widenmaier *et al.*, 2017). This illustrates that Nfe2l1 displays multiple functions and several layers of transcriptional and post-translational regulation, all of which require further investigation.

Summary and outlook

While a large effort has been directed at understanding the molecular basis of proteostasis through studying UPR and autophagy, much less is known about the role and regulation of UPS/ERAD, particularly under physiologic conditions. Only now we are realizing that in our body different cell types have different approaches to maintaining proteostasis and that with this there are multiple layers of regulation linked to human physiology and pathophysiology. Nfe2l1 is a new key player and its pathway is interconnected within the proteostatic relays of the cell. The unique biology and relevance of Nfe2l1 for obesity-associated metabolic disorders raises the hope that within the machineries of ERAD and UPS there are additional

new approaches for developing novel therapeutics for the modern-day global crisis of obesity-linked diseases.

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Conflict of interest statement

The authors declare no conflict of interest.

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Figure Legends

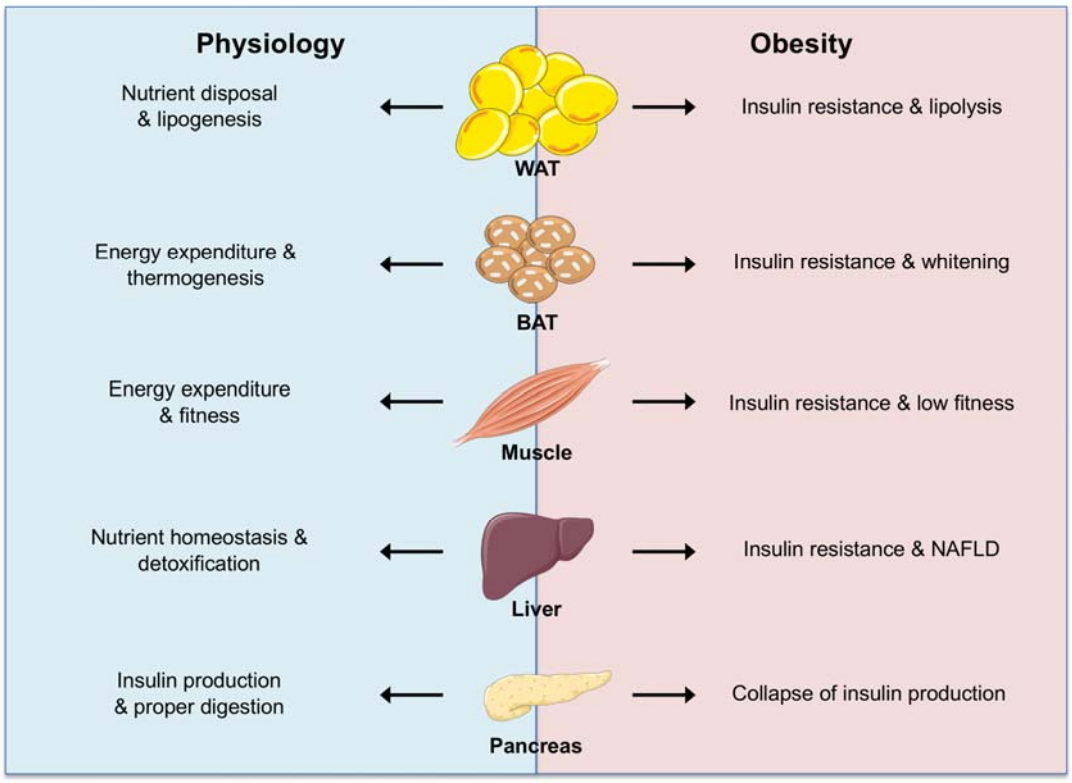

Figure 1 Function and dysfunction of metabolic tissues.

The physiologic roles of the tissues that most critically regulate metabolism support the portioning of nutrients. However, in obesity these responsibilities are disturbed and insulin resistance as a main driver of dysfunction ultimately results in metabolic disease.

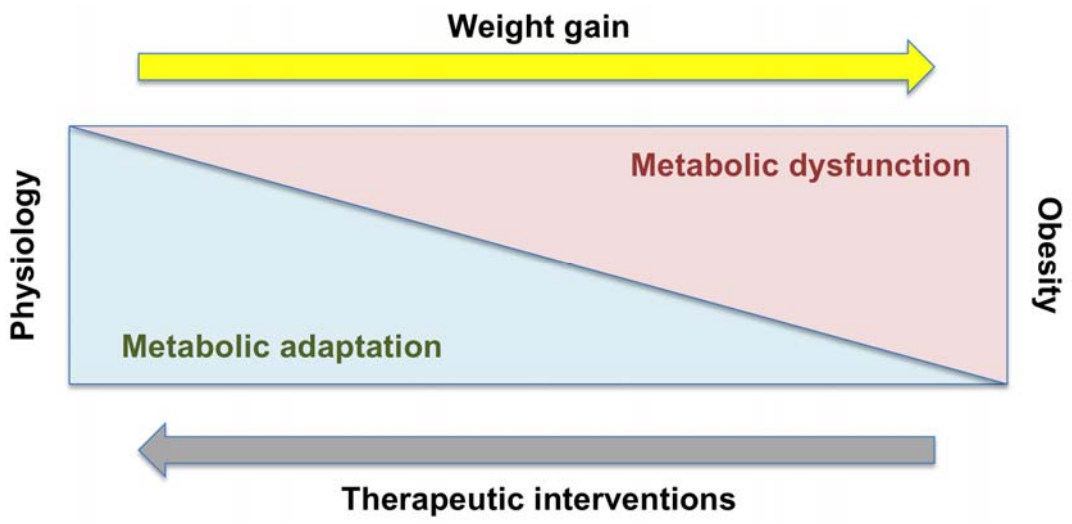

Figure 2 Metabolic adaptation and dysfunction.

When energy intake exceeds energy expenditure excess nutrients induce weight gain. While there are programs in place that mediate the healthy adaptation of metabolism during the initial phase of weight gain, prolonged metabolic stress causes these adaptive programs to eventually fail in obesity.

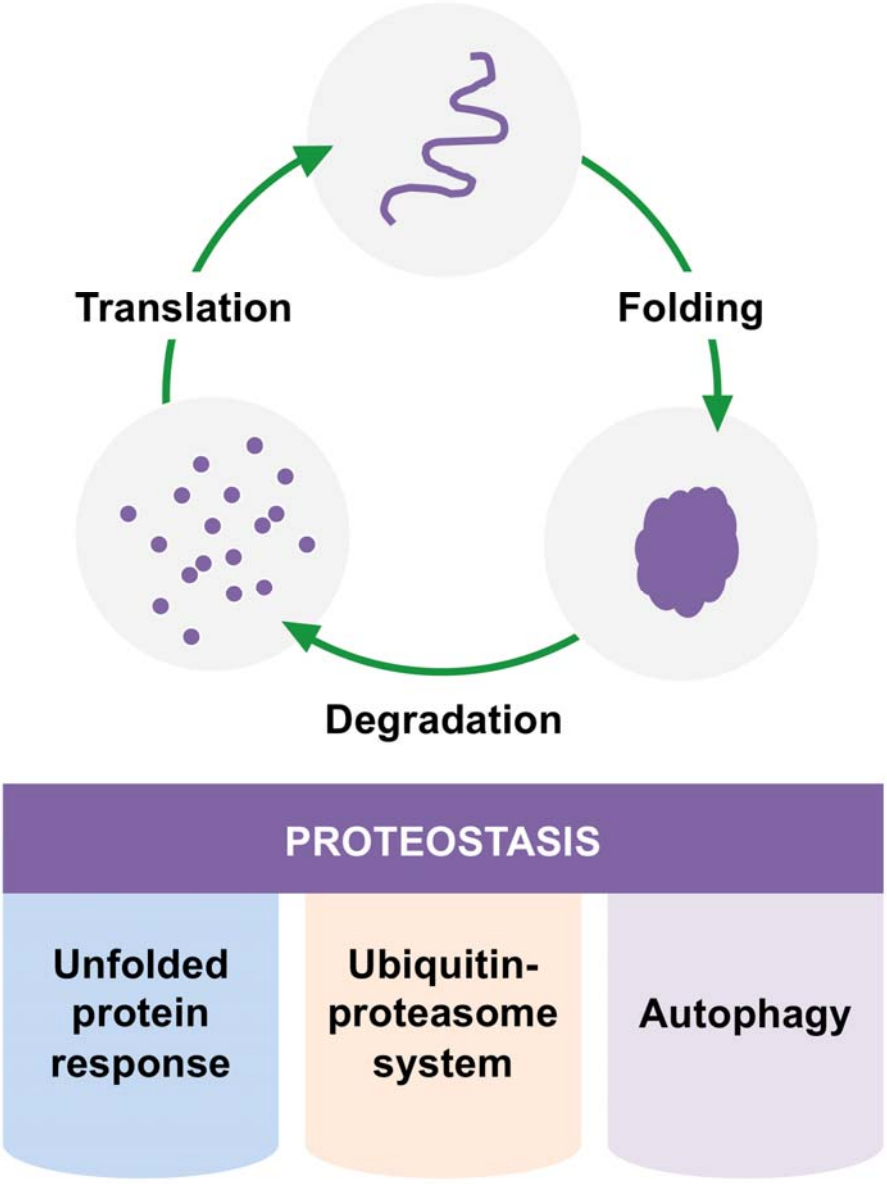

Figure 3 The pillars of proteostasis.

The life cycle of proteins is regulated by quality control mechanisms to sustain proteostasis. The unfolded protein response (UPR), the ubiquitin-proteasome system (UPS), and autophagy are interdependent programs working together to ensure cellular function and adapting the proteome to environmental challenges.

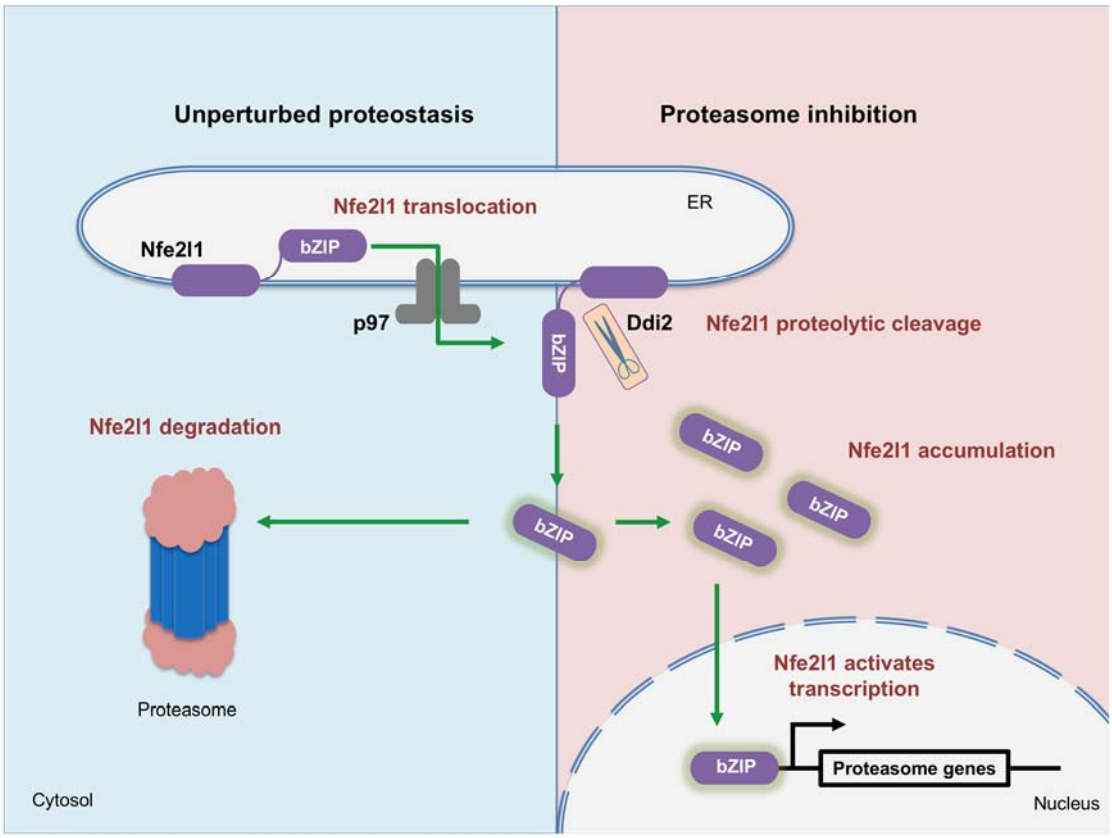

Figure 4 Adaptive proteasomal activity mediated by the bounce-back Nfe2l1 mechanism.

In a simplified model derived from experiments with proteasome inhibitors in cancer cells, the ca. 120 kDa Nfe2l1 resides in the endoplasmic reticulum (ER) membrane with its transcription factor moiety (bZIP) in the ER lumen. The translocase p97 shifts Nfe2l1 across the membrane, where the 95 kDa bZIP-containing domain is proteolytically cleaved off the anchoring protein remnant by the protease Ddi2. Under normal homeostatic conditions with unperturbed proteostasis, this 96 kDa is thought to be rapidly degraded by the proteasome. Now, when proteasomal activity is blocked by chemical proteasome inhibitors, the 95 kDa-fragment accumulates and travels to the nucleus to bind to promoters of proteasome genes, including the *Psm* subunit genes.

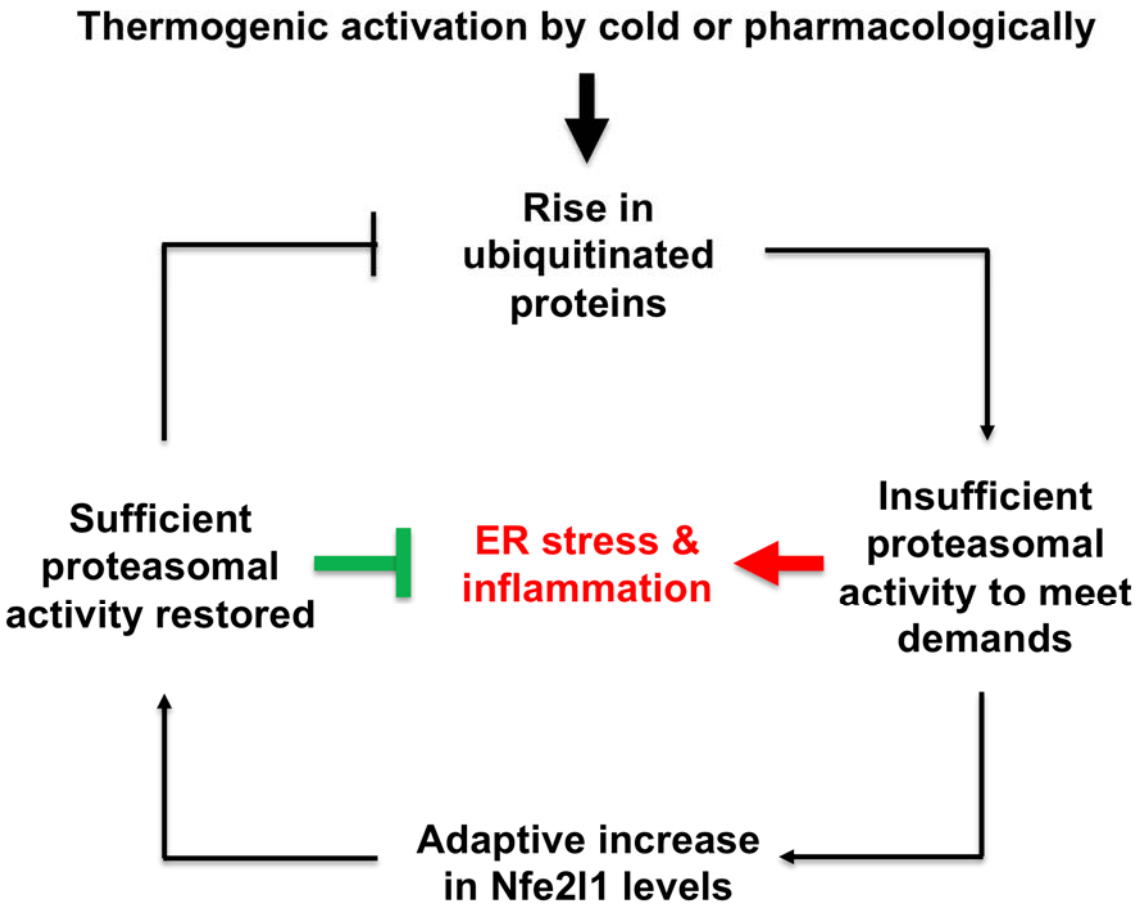

Figure 5 Role of Nfe2l1 in adapting proteostasis in thermogenesis.

The activation of thermogenesis and its increased metabolic rate represents a challenge to proteostasis. Cold adaptation naturally increases the levels of ubiquitinated proteins, which requires matching proteasomal activity to increased demands. This is facilitated by the transcription factor Nfe2l1 that transcriptionally induces the production of more proteasome to restore proteostasis. If cells fail to adapt proteasomal activity the consequences are accumulation of hyperubiquitinated proteins, ER stress and inflammation.