Abstract: Non-communicable diseases (NCDs) such as cardiovascular disease, cancers, diabetes and obesity are responsible for about two thirds of mortality worldwide, and all of these ailments share a common low-intensity systemic chronic inflammation, endoplasmic reticulum stress (ER stress), and the ensuing Unfolded Protein Response (UPR). These adaptive mechanisms are also responsible for significant metabolic changes that feedback with the central clock of the suprachiasmatic nucleus (SCN) of the hypothalamus, as well as with oscillators of peripheral tissues. In this review we attempt to use a systems biology approach to explore such interactions as a whole; to answer two fundamental questions: (1) how dependent are these adaptive responses and subsequent events leading to NCD with their state of synchrony with the SCN and peripheral oscillators? And, (2) How could modifiers of the activity of SCN for instance, food intake, exercise, and drugs, be potentially used to modulate systemic inflammation and ER stress to ameliorate or even prevent NCDs?

Keywords: Systems biology, inflammation, circadian, oscillators, metabolism, communicable diseases, UPR, stress

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1 Introduction

Non-communicable diseases (NCDs) are responsible for about two thirds (65.5%) of worldwide mortality. By 2010, just three ailments accounted for 64.35% of all mortality by NCDs, namely, cardio- and cerebrovascular disease, cancers, and diabetes [1]. Such diseases are preventable by a host of lifestyle measures that include large-scale control of tobacco and alcohol consumption, comprehensive changes in dietary intake of sugars, salt, harmful fats, and increasing daily consumption of fresh fruits and vegetables. These, and additional preventive measures such as early clinical interventions, may help to significantly reduce the risk to NCDs [2].

Prevention of NCDs, in particular the three major diseases referred above, and additionally obesity, is of paramount importance given the close pathophysiological relationship between them [3]. What seems to link them all are interlocked complex networks [4,5], controlling signal transduction of adaptive responses such as the unfolded protein response, UPR [6,7], systemic chronic inflammation [8], and the central clock of the suprachiasmatic nucleus (SCN) of the hypothalamus [9-11].

Through systems biology it is now possible to get a preliminary understanding on how such interplay of biological information is organized into complex networks transducing signals from a host of environmental stimuli into adaptive responses that could potentially end in systemic chronic inflammation and disease [12, 13].

Using the system biology approach, the networks can be understood as organizing into stacks in hierarchical order; of numerous functional modules controlled by few nodes (hubs), made of key regulator molecules connecting every module through a myriad of positive and negative feedback loops [14, 15].

These networks can be probed to study in detail how the signal transduction machinery connects, for instance, energy metabolism and the systemic chronic inflammation preceding most if not all NCDs [12, 13, 16]. In this respect, in the last few years many groups of researchers have uncovered ample evidence pointing to energy metabolism as a key link to the UPR and inflammation, with metabolic risk factors that may eventually lead to some of the clinical manifestations of the big four NCDs [7, 17, 18].
Metabolic risk factors for NCDSs include high blood levels of triglycerides (TG), low levels of high-density lipoproteins (HDL), high blood pressure, impaired fasting glycaemia, abusive alcohol ingestion, tobacco, physical inactivity and excess food intake. Additional risk factors include elevated circulating inflammatory markers (i.e. C-reactive protein, tumor necrosis factor, interleukin-6), and reduced levels of anti-inflammatory hormones such as adiponectin [19-22].

Another major development with practical applications from the information systems underlying such physiological and biochemical phenomena, is the realization that energy metabolism is tightly regulated by a web of biological oscillators distributed in peripheral tissues, and entrained by environmental changes that follow a circadian rhythm dictated by the SCN [10, 23-25].

In this review we will attempt to use a systems biology approach to explore such interactions as a whole to get at least some partial answers to two fundamental questions regarding the role of ER stress on disease: [1] how dependent is the UPR and subsequent events referred above, to its state of synchrony with the SCN and peripheral oscillators? And, [2] How could modifiers of the activity of SCN, (for example, by means of changing food intake, exercise, and drugs) be used to modulate ER stress and systemic inflammation to ameliorate or even prevent NCDSs?

In answering the above questions, we will first delve into the relationships between chronic inflammation, the ER and the UPR, and NCDSs. Each of these topics will be discussed, with specific examples relating to common NCDSs, including heart disease, diabetes, and cancers. Out analysis will begin broadly; the first part of the review will be devoted to the inflammatory response and NCDSs. The review will then narrow into an in-depth discussion of the progenitors of chronic inflammation, namely ER stress and the subsequent UPR pathway. Following that, we will again broaden the discussion to show the crosstalk between the UPR, the SCN, and peripheral oscillators. Finally, we will link chronic inflammation, ER stress and the UPR, and oscillators, concluding with a discussion on the potential implications for treatment of NCDSs as discovered through the systems biology framework.

2 The link of NCDs to Systemic Chronic Inflammation

A considerable body of evidence accumulated in the last twenty years shows a causal relationship between the onset of major NCDs with networks of the inflammatory response, both at the epidemiological, clinical and experimental levels. Although inflammation is a physiological response to limit bodily harm to many types of aggressions, when deregulated or chronic, may become collateral damage and immune pathological to the host [26]. Thus systemic chronic inflammation appears to be linked to homeostatic disequilibria (i.e. tissue stress) of one or more networks of adaptive response, which are not necessarily related to the classical aggressors of host defense or tissue repair (i.e. infections and tissue damage). This is the case in cardiovascular disease, CVD [12], cancers [3], type 2 diabetes and obesity [8].

Perhaps the earliest convincing evidence of this homeostatic disequilibrium was the demonstration in 1993, of the overexpression of tumor necrosis factor-α (TNF-α) in adipose cells of obese and diabetic experimental animals [27]. Tumor necrosis factor-α is capable of altering glucose homeostasis by targeting and inhibiting GLUT4 translocation to the plasma membrane by tissue-specific mechanisms [28]. Furthermore, adipocytes from obese animals communicate their stress condition to neighboring macrophages by secreting monocyte chemoattractant protein–1 (MCP-1), resulting in macrophage infiltration into adipose tissue and augmented hepatic fat accumulation. These effects can be significantly reduced in MCP-1 homozygous KO mice when compared with WT animals [29]. These results are, therefore, consistent with the role of tissue-resident macrophages as monitors of cell stress to maintain homeostasis [30].

These cell-cell interactions of adipocytes with tissue-resident macrophages, via adipokines or adipocytokines and cytokines, can become maladaptive and produce collateral damage in the form of insulin resistance in adipose tissue. Such communications can also cause a wide range of systemic effects of inflammation leading to a vicious circle of more obesity and type 2 diabetes [26]. Adipokines other than MCP-1 and TNF-α, cover a wide range of chemical mediators, amid them interleukin-6 (IL-6), leptin, resistin, adiponectin, plasminogen activator inhibitor (PAI-1), visfatin, apelin, omentin, and nefastin, among others [12]. The production of such bioactive mediators would explain the close association of systemic chronic inflammation with obesity, and also with cardiovascular disease [31].

The role of systemic chronic inflammation on CVD, with macrophages as protagonists, has been well documented for common ailments such as atherosclerosis, myocardial infarction and heart failure [32]. In short, the involvement of inflammation in CVD starts very early on with the retention of Apo lipoprotein (Apo) B-containing
lipoproteins by the intima of arteries in regions of disturbed blood flow. Such lipid accumulation prompts the infiltration of monocyte-derived macrophages to phagocytize the lipid moieties by virtue of a pattern recognition mechanism, embedded in specific receptors of the plasma membrane (pattern recognition receptors, PRRs), which targets different native and oxidized lipoproteins for lysosomal degradation. Furthermore, there is a relay of the inflammation response mediated by pattern recognition receptors, among them the nucleotide-binding oligomerization domain receptor, or NOD-like receptors (NLRs). That type of mechanism mediated by PRRS occurs with NLRP3-inflammosomes that respond to cholesterol crystals, and release a powerful inflammatory cytokine, interleukin-1β (IL-1β).

Inflammosomes are key elements of the innate immune response, and they consist of multiprotein complexes of the intracellular milieu that are capable of detecting and responding to many inducers of inflammation, such as the wide range of pathogen-associated molecular patterns (PAMPs), as well as the damage-associated molecular patterns (DAMPs), which cause tissue damage due to metabolic imbalances. The structure of Inflammosomes is made of three components, a sensor molecule, the adaptor protein ASC, and caspase-1, being the former a PRR member of the NLR family (i.e. NLRP3 referred above) that recruits the adaptor protein ASC, which in turn ligates and activates caspase-1. The activation of the latter is then responsible for both the maturation of the cytokines IL-1β and IL-18, and a form of cell death induced by inflammation called pyroptosis [33, 34].

The occurrence of systemic chronic inflammation in CVD can therefore, be used to predict risk of the disease, by examining of key signaling mediators such as the blood levels of C-reactive protein (CRP). This molecule is an early indicator of systemic inflammation, synthesized mostly in the liver, but regulated by other cytokines such as blood levels of IL-6, and by TNF and IL1 [35]. Epidemiological studies, as well as a meta-analysis of research comprising hundreds of thousands of subjects indicate that CRP blood levels can be a strong predictor of cardiovascular risk [36, 37].

The close relationship of systemic chronic inflammation with other major NCDs such as many types of cancers is also well established, both at the epidemiological, clinical and the molecular levels [38-40]. Many studies have thus demonstrated the presence of inflammatory mediators in several kinds of cancerous tissues, or in the increase of cancer risk (colon, prostate, pancreas) in subjects with chronic inflammation triggered by different inducers such as infectious agents and tissue damage. The net effect of either pathway is the recruitment and infiltration of neoplastic tissue by inflammatory cells (i.e. macrophages, mast cells, eosinophils), and the activation of hubs of proinflammatory networks, such as NF-kB, STAT3, and HIF1α, resulting in further infiltration of cancer tissues by inflammatory cells [41].

The inflammatory process can thus aggravate the neoplastic state with more cell proliferation, tumor cell migration, invasion, angiogenesis and metastasis, the inhibition of adaptive immunity, and hormonal disequilibria [38]. Thus, as with the other NCDs mentioned previously, cancer exhibits a vicious circle whereby locally initiated events of cell-cell communication of stressed cells may lead to the amplification of cancer-initiated inflammation [40].

The adaptive immune system, however, also plays a key role as surveyor and killer of nascent tumors by virtue of a mechanism called immune editing. This consists of a cancer immunosurveillance network that is capable to detect tumor-specific markers and eliminate developing cancers and tumor formation. The neoplastic cells, however, may resist and remain in an equilibrium phase, or be “molded” by immune “editors” and therefore produce tumor variants that may eventually evade the immune system, which result in clinically detectable tumors in the scape phase [42]. This sort of dual function of the immune system may help to resolve the apparent paradox of the role of systemic chronic inflammation in enabling or suppressing cancer development [40].

Systemic chronic inflammation, microbiota and NCDs

Another mechanism related to environmental factors by which systemic chronic inflammation can be triggered or modulated is through the microbiota of the gastrointestinal (GI) tract. The microbiota seem to affect not only cancer development, but also obesity, type 2 diabetes, and CVD as well. The processes involve the role of diet and nutrition on the resident microbiota of the gastrointestinal tract and other body sites [16, 43-47].

The microbiota and their corresponding genomes, collectively termed microbiome, can be quantitatively assessed by the use of modern sequencing platforms and their respective bioinformatics systems, which are focused primarily on 16S ribosomal RNA [48]. The microbiome of the GI tract, for instance, is very sensitive to a host of environmental factors; in particular diet [49], age and geography [50, 51], antibiotics [52], and other chemotherapeutic agents [53, 54], as well as many other factors that add to the complexity of environmental interactions [55, 56]. It is not surprising that disturbances of the microbiota, termed dysbiosis [43], are associated
with NCDs such as cancers [43, 44, 54], obesity [45, 46, 57], type 2 diabetes [58], and, of course, to the systemic chronic inflammation related to such diseases [16, 47, 59-61].

The mechanisms by which systemic chronic inflammation is related to NCDs through changes in the microbiome is as follows. The GI tract microbiome is responsible for a gentle balance of pro- and anti-inflammatory processes that are critical to maintain gut immune homeostasis. Among such mechanisms are the effects of metabolic by-products on the immune system to modulate peripheral regulatory T cell generation. These involve mutualistic cell-cell communication via bacterial metabolites, such as short-chain fatty acids (SCFA), butyrate and propionate, which are capable of potentiating extrathymic differentiation of regulatory T cells that play a fundamental role in suppressing inflammatory and allergic responses [59, 62].

Similarly, resident microbes of the gut can also stimulate B-cell development and antibody diversity in the intestinal mucosa, by a mechanism involving receptor editing of immature B cells mediated by their interaction with non-pathogenic microbes in the gut lumen [63]. The net effect is the induction of B-cell tolerance to such resident microorganisms, and the increase of the repertoire of B-cell receptors [64].

From the above discussion it follows, that any change in the microbiota of the GI tract, induced by either environmental factors (diet, infections, of any other forms of stress inducers) or from endogenous sources (i.e. disturbances of the intestine integrity) would, have an impact on the immune response of the host, which would include systemic inflammation [65]. It is not surprising that changes in the microbiota composition, correlate well with several types of cancers such as colorectal, pancreatic, gastric, gall bladder, oral squamous cell carcinoma, and esophageal cancer [43].

Perhaps the best examples illustrating the nature of the relationship of neoplastic tumors with GI microbiota, are gastric ulcers, gastric cancer, and Helicobacter pylori [66]. Roughly 50% of the world’s population harbors this bacterium, and while this particular microorganism is benign for most people, rarely gastric cancer can be developed as a result of the action of the bacterium [67, 68]. Carcinogenesis by H. pylori, involves the hijacking of tumor suppressor p53, mediated by cytotoxin-associated gene A (CagA) of type I strains of H. pylori. Cytotoxin CagA then binds to the apoptosis-stimulating protein of p53 (ASPP2) inside the host cells, and recruits its natural ligand p53 to inhibit its apoptotic function, and promote cell survival and transformation in an ASPP2-dependent fashion [69].

Another example is colorectal cancer, where many bacterial species can contribute to colitis-associated adenomas and carcinomas by a combination of secretion of microbial toxins, and/or disturbance in the colonic epithelial barrier. Among these bacteria are Streptococcus gallolyticus, adherence- invasive strains of Escherichia coli, enterotoxigenic Bacteroides fragilis, and Fusobacterium nucleatum [65, 68].

The microbiome can also affect obesity, as shown by transferring donor’s microbiome communities from separated twin pairs of mice, where those receiving an obese twin’s fecal microbial population displayed a greater body mass than mice receiving microbial from the lean twin’s gut. Moreover, in a diet-dependent fashion, cohousing obese and lean animals could prevent adiposity. Members of the phylum Bacteriodetes were identified as successful rescuers from the lean microbiota into the obese microbiome, although no cause-effect was proven [45].

Dietary-induced changes of the gut microbiota can also relate obesity with cancer in mice by the production of secondary bile acids such as the microbial metabolite deoxy-cholic acid (DCA), a known cause of DNA damage [70]. The enterohepatic circulation of DCA in mice eventually leads to a series of inflammatory mediators and tumor-promoting factors in the liver, thus making the tissue highly vulnerable to chemical carcinogens and further development of hepatocarcinoma. Interestingly, blocking DCA production or reducing gut microbiota in obese mice can prevent such type of carcinogenesis [46].

Obesity has previously been shown to alter the cecal microbiome ecology, at least in experimental models of obese mice, which have shown a reduction of the abundance of Bacteriodetes and a related increase of Firmicutes, the two most abundant groups microbes at the phylum level in both mice and humans [57].

In humans, a recent study could not find any phenotypical correlation between body adiposity and the ratio of Firmicutes to Bacteriodetes [71]. Nonetheless, there seems to be sharp differences in the richness of the metagenome of lean individuals when compared with those with adiposity, insulin resistance, dyslipidemia, and a manifested inflammatory phenotype that was characterized with a low bacterial richness in contrast to healthy individuals [60]. These results are consistent with the moderate microbial dysbiosis of Chinese subjects with type 2 diabetes characterized by a low abundance of butyrate-producing bacteria, and an enrichment of microbial functions related to oxidative stress resistance [72].
3 Systemic chronic inflammation, ER stress and NCDs

The inflammatory response and ER stress intersect to maintain homeostasis, along with other important adaptive mechanisms. When such crosstalk goes astray it may lead to disequilibria, more systemic chronic inflammation, the metabolic syndrome, and possibly the onset of chronic disease [73]. The ER is a major site for sensing cellular stress given the highly sensitive UPR, finely coupled to protein synthesis and protein processing (post-translational events, folding by chaperones, and oligomerization), quality control, autophagy, and vesicular trafficking. The ER is also a site of lipid synthesis including sterols, and the storage of key mediators of signal transduction such as free calcium [17, 74].

The signal transduction mechanism involves three major avenues of adaptive response [see Figure 1], termed collectively the UPR, consisting of transcriptional and/or translational programs that increase the capacity of the ER to accommodate changing protein demands, and/or cell aggressions, to initiate a systemic adaptive response to cellular stress [17]. Such responses include a succession of physiological processes that promote the anti-oxidative response, apoptosis, and over time, systemic chronic inflammation [75].

Systemic chronic inflammation and many of the NCDs are fundamentally entwined with the UPR signaling and metabolic processes [73]. An excess of metabolic products from hyperlipidemia, for instance, can induce the UPR by an overload of unesterified cholesterol in the ER membranes, and deplete internal ER calcium stores [76]. Similarly, saturated fatty acids such as palmitate and stearate (which is very common in a western diet) can also induce ER stress and promote apoptosis in liver cells by the accumulation of ceramide [77].

Another example of ER sensitivity to metabolic products is glucose homeostasis, which in diet-induced obese mice is particularly dependent on the functioning of the ER in beta cells. Thus a sustained hyperglycemia can affect the translation of proinsulin, and perturb the secretion capacity of the ER resulting in the induction of systemic chronic inflammation.

![Figure 1: Molecular mediators of the UPR. Schematic representation of the two successive stages of the UPR where the upper panel depicts the three major branches, PERK, IRE1, and ATF6, and their corresponding gene targets, transcription factors, ATF4, XBP1, and ATF6(N). The lower panel shows the physiological adaptive processes associated to each branch of the UPR and their respective transcription factors.](image-url)
the UPR. It thus appears that insulin synthesis may be coupled with the folding capacity of the ER, and that the resulting signaling might also be a key factor to prevent diet-induced type-2 diabetes [78].

All, if not most, of such crosstalk between key metabolites and the UPR in different tissues, results in the production of elevated levels of pro-inflammatory mediators such as cytokines and adipocytokines [6, 7, 73, 79]. Among these are TNFα, IL6, and IL1β that would increase chronic inflammation of hepatocytes, pancreatic β cells, and adipocytes, respectively, as well as in macrophages [8].

In atherosclerosis there is prolonged ER stress and activation of the UPR in endothelial cells, which is induced by oxidative stress as well as increased levels of intracellular cholesterol and saturated fatty acids. Other inducers of ER stress are LDL modification, which is caused by a variety of processes including oxidation, lipid hydrolysis, and protein glycation [80].

ER stress of endothelial cells also occurs in diabetes, and the most probable cause may be the accumulation of glucosamine in the cells due to hyperglycemia [81]. Another inducer of ER stress in endothelial cells might be homocysteine, given the apparent role of hyperhomocysteinemia in cardiovascular disease, which would include stroke, peripheral vascular disease and ischemic heart disease [82, 83].

Atherogenesis, therefore, seems to follow the usual vicious circle of other NCDs, compounded by ER stress and systemic chronic inflammation, and mediated by signaling from infiltrating macrophages, dendritic cells, T cells, mast cells, B Cells and endocytes [84]. It is important to note that ER stress may induce macrophage apoptosis, and that seems to be a relevant event in the generation of necrotic cores and the subsequent accumulation of dangerous plaques [85]. These events together, when sustained, may lead to systemic chronic inflammation by affecting the whole cardiovascular system [86], hepatic lipid metabolism, [87] and pancreatic beta cell function [78].

In cancer, cell proliferation may induce ER stress and the UPR by a variety of stimuli from the microenvironment, such as glucose deprivation, oxidative stress, decreased amino acid supplies, and hypoxia, as well as by perturbances in glycoprotein and lipid biosynthesis due to mutations [88]. In addition, the UPR becomes a source of proinflammatory mediators that may compromise the antitumor immunity [89].

The relationship of ER stress with cancer formation, appears to be especially intriguing given the apparent paradox posed by a possible preventive effect of the UPR through its apoptotic pathway or, to the contrary, a deleterious outcome resulting from tumor development under taxing conditions of the microenvironment. The paradox is somewhat explained by the duration and severity of ER stress, thus at the early stages the ensuing UPR protects the stressed cells from uncontrolled proliferation and tumor formation, whereas in prolonged cell stress, two of the three major avenues of the UPR [see next section, Figures 1 and 2] may bypass apoptosis and favor tumor formation [90].

4 Molecular pathways of the UPR

The UPR is basically a signal transduction mechanism from the ER to the nucleus activating a host of transcriptional pathways to maintain homeostasis, which syncs with other key signaling networks [91]. At the molecular level, the three branches of the UPR consist of transmembrane proteins that become activated as ER sensors at different stages throughout the cellular stress.

The early physiological response consists of a reduction of the influx of proteins into the ER to recover homeostasis, carried out by a protein kinase, PERK (double-stranded RNA-activated protein kinase), with a strong effect on arresting overall protein translation. A multifunctional enzyme supplements this action, IRE1 (inositol requiring enzyme 1, both α and β isoforms) that is able to degrade the specific mRNAs encoding certain ER-located proteins. Finally, at a later stage ATF6 intervenes (activating transcription factor 6, both α and β isoforms), a master regulator of transcription of the UPR target genes [17, 92]. At this point misfolded proteins become targeted and degraded by the ubiquitin-proteasome system, which is activated by the transcriptional induction of components of ER-associated degradation, ERAD, [93].

The mechanism of activation of the UPR goes as follows. Unfolded proteins in the lumen of the ER trigger the three respective protein sensors by dimerization (PERK), oligomerization (IRE1), or proteolysis (ATF6). These will induce the respective transcription factors ATF4, XBP1 (X-box binding protein), and ATF6n that will enter into the nucleus to activate transcription of the target genes of the UPR. This mechanism increases the protein folding capacity of the ER.

In addition, branches IRE1 and PERK also decrease the protein load in the lumen, via RIDD (regulated IRE1 dependent decay) and phosphorylation of eIF2a (translation initiation factor), respectively. Another pathway prompted by IRE1 is the activation of autophagy by its interaction with JNK (JUN N-terminal kinase). If such
adaptive mechanisms could not reestablish homeostasis upon continued ER stress, then apoptosis would follow to eliminate unrepaired cells [17,92].

The mechanisms by which every protein sensor activates transcription of their corresponding target genes are distinct. In the early UPR, in response to unfolded proteins in the lumen, PERK dimerizes and phosphorylates itself by an intramolecular reaction, and then inactivates eIF2a by phosphorylation and inhibits mRNA translation [17]. This alleviates ER stress by reducing protein synthesis, but some mRNAs getaway, resulting in their indirect induction by preferential translation. Among these mRNAs are ones that encode for transcription factor ATF4, which then drives the induction of CHOP (transcription factor C/EBP homologous protein), a very important gene product controlling apoptosis [94]. It follows, then, that the PERK branch of the UPR can have a dual role, of protection or cell death, depending of the level of signaling from ER stress.

Another interesting feature of the signal transduction mediated by PERK/ATF4/CHOP has to do with providing a mechanism to counterbalance its own action by inducing GADD34 (growth arrest and DNA damage-inducible 34). The gene encodes a regulatory subunit of PP1C, a protein phosphatase that can reverse the inactivation of eIF2a. In cells with protein misfolding stress, the administration of small molecules could help to restore homeostasis by selective inhibition of the phosphatase GADD34-PP1c; this would prolong eIF2 phosphorylation in stressed cells, giving enough time for the cells to adjust translation to their levels of accessible chaperones [95,96].

The signal transduction mechanism by IRE1, an enzyme with a dual function of protein kinase/endoribonuclease, follows an entirely different route from the one of PERK. The UPR activates IRE1 by its oligomerization in the ER membrane involving trans-autophosphorylation, and further activation of its ribonuclease (RNase) activity. The latter then cleaves the mRNA of XBP1, a UPR specific target, in two exact positions removing an intron. The net result is a spliced mRNA translated into the active form of the transcription activator XBP1s [17,97,98]. XBP1s upregulates the gene transcription of target proteins involved in folding and protein quality control, ERAD.

XBP1 also regulates lipid biosynthesis, and it is involved in the convoluted ER typical of active secretory cells [99]. In addition, both kinase and RNase activities of IRE1 are required for splicing the mRNA of XBP1s leading to activation of the UPR. Moreover, IRE1 phosphorylation is essential for RIDD activity as well as recruiting other target protein molecules such as TRAF2 (TNF receptor associated factor 2) [100].

The third branch of the UPR entails the precursor of yet another transcription factor, ATF6. Upon accumulation of unfolded proteins dissociates from its chaperone BiP/GRP78 (binding immunoglobulin protein/glucose regulated protein), ATF6 translocates from its transmembrane location in the ER to vesicles pinched off the ER and delivered to the Golgi apparatus [101].

In the Golgi, ATF6 is clipped by two proteases, SIP and S2P (site-1 and site-2 protease) that liberate the terminal cytosolic fragment ATF6(N) that migrates to the nucleus to act as a master regulator of UPR target genes [102]. Several chaperones are activated this way, in particular BiP/GRP78 and glucose-regulated protein 94, GRP94 [17,103]. The mechanism of activation involves recruitment by ATF6 of a collection of RNA polymerase II (Pol II) coregulatory complexes, which bind UPR-specific enhancer elements in their promoters.

The Pol II coregulatory complexes encompass the Mediator (a multi-subunit assembly essential as a scaffold for Pol II mediated transcription) and several histone acetyltransferases (HATs), such as transcriptional coactivators Spt-Ada-Gcn5 acetyltransferase (SAGA), and Ada-Two-A-containing (ATAC) complexes [104]. SAGA acetylates histone H3 (H3K14), and seems to play a dual central role in the ER signaling mechanism. On one hand, prior to ER stress it keeps a “poised” chromatin state on target gene promoters; on the other hand, upon induction of ER stress this enzyme becomes essential for the H3K14 acetylation of such genes, resulting on transcriptional activation and survival of human cells [105].

ATAC complexes also acetylate lysine 14 of H3 histones, but execute their coactivating function on distinct sets of UPR target genes. As is the case with SAGA, ATAC complexes display a dual function on ER stress gene promoters; the enzyme essential for the induction of target genes after stress, but under basal conditions it is required for maintenance of the low levels of H3 acetylation marks of these promoters. Interestingly enough, ATAC complexes also display a strong effect on the overall phosphorylation of histone H3S10, which plays another important role in the transcriptional regulation of genes targeted by ER stress [106].

5 The integrated UPR: cell adaptation or death

The branches of the UPR control the recovery of protein homeostasis, and/or the decision to undergo cell death by apoptosis if the adaptive mechanisms of the UPR fail upon unmitigated ER stress. Although all branches contribute to either survival or apoptotic activities, as illustrated
in Figure 2, there is controversial evidence regarding the respective molecular mechanisms for each branch of the UPR. It appears that PERK is the direct switch to apoptosis, whereas IRE1 controls the balance of most of the survival genes and protein homeostasis recovery. Under demanding circumstances, however, both branches will work simultaneously to induce apoptosis [107].

Two interconnected circuits can lead to apoptosis, the mitochondrial or intrinsic pathway, and the extrinsic route mediated by death receptors (DR4/5, TNFR1, CD95). Both pathways involve specific proteases called initiator caspases (caspase 2 for the mitochondrial, and caspase 8 for the extrinsic pathway) that activate a set of common executioner caspases [108, 109].

The branch ATF4/CHOP pathway of the UPR may promote the core mitochondrial pathway of apoptosis under unmitigated ER stress through several steps, including: downregulating the antiapoptotic BCL2 protein (B-cell lymphoma-2), and by upregulating the gene expression of TRB3 (tribles-related protein3), DOCs (for downstream of CHOP), GADD34, and the gene of BIM [Bcl-2-interacting mediator of cell death], which sensitizes the cells for cell death [110]. In addition, overexpression of ATF4 and CHOP can boost protein synthesis to cause ATP depletion, oxidative stress, and cell death [111].

Under sustained ER stress, IRE1 also intervenes to induce apoptosis by derepressing the translation of caspase-2 expression arresting microRNA biogenesis [112]. However, a recent report casts serious doubts on the UPR induction of apoptosis by the caspase-2 mitochondrial pathway, because in deficient mice there were no significant impact of the loss of caspase-2, on ER-stress induced apoptosis [113].

This controversy might be resolved by the fact that sustained ER stress is capable of promoting apoptosis by the extrinsic pathway mediated by DR5 (Death Receptor 5) induced by CHOP, although IRE1 did catalyze DR5 mRNA decay, allowing time for recovery of protein homeostasis. As the exposure to ER stress persisted, there was a buildup of DR5 protein driving apoptosis via caspase-8 [114].

The CHOP mediated pathway, therefore, may not be sufficient in itself to drive apoptosis apart from the evidence referred above, overexpression of CHOP alone does not induce apoptosis [115]. A different and additional mechanism may exist to promote apoptosis downstream of the activation of PERK and eIF2 phosphorylation, which may consist of PERK mediated suppression of XIAP (X-linked inhibitor of apoptosis protein). The evidence further shows that the down-regulation of XIAP is independent of CHOP activity, and that the loss of XIAP increases cell death [107].

Thus it may be possible that the involvement of PERK in cell fate encompasses a two-stage process, by which cell survival will initially be favored by the activation of PERK, with further induction of CHOP until protein homeostasis is restored. Under unresolved ER stress, a second stage will switch the cell fate to apoptosis, by simultaneous up-regulating CHOP and suppressing XIAP, both under the control of PERK [107].

Finally, the action of ATF6 as the master regulator of the UPR target genes, as depicted schematically in Figure 2, optimizes key functions of the ER such as protein folding, secretion, and protein degradation during cell stress, favoring survival [116]. ATF6, however, can also induce cell death after sustained ER stress, although the exact mechanism for the latter is still unresolved. The recovery of protein homeostasis after ER stress by ATF6 also includes induction of transcription of chaperones Bip/GRP78 and GRP94, as mentioned above, and the upregulation of gene product XBP1, the target of IRE1. Under persistent ER stress, however, ATF6 could lead to apoptosis via CHOP [117], and in certain cell systems it may drive cell death by a mechanism involving the reduction of the antiapoptotic protein, Mcl-1 (myeloid cell leukemia sequence 1) during myoblast differentiation [118].

6 Endoplasmic reticulum stress and the circadian clock of the SCN

The circadian clock influences almost all aspects of biology including the UPR, and it may come to no surprise that about half of all human transcripts are subjected to circadian oscillations at the mRNA level [119]. The links of the UPR to the circadian clock are both downstream of the two major executor branches, PERK, and IRE1, and on the other hand, upstream to the master gene regulator, ATF6 [120].

The molecular components of the SCN neurons, and peripheral cells such as retina, the liver, and intestine, among other tissues, consist of transcription-translation feedback loops made of gene activators, coactivators, and repressors that cycle synchronously with the central clock according to a 24-hour period [23].

The SCN, fine-tunes entrainment by day-light and consumption of food, with a variety of physiological functions that combine direct clock outputs affecting body temperature, blood pressure, glucose homeostasis, appetite, energy outlay, behavior and sleep/wakefulness [121, 122]. The SCN also coordinates the functions of peripheral oscillator’s outputs, regulating a wide range of key metabolic activities such as food absorption,
hormonal secretion and signaling, fat accumulation, lipogenesis, and glucose tolerance [23, 24, 123].

Figure 3 depicts this complex array of relationships between the SCN (entrained by light and food), and the downstream events triggered by the release of hypothalamic hormones controlling energy metabolism (ACTH, TSH, GH), reproduction (FSH), and blood pressure (vasopressin) [25].

At the molecular level, two major circuits of gene products are responsible for the control by the SCN of circadian rhythm in mammals, as depicted in Figure 4. At the core are the transcriptional activators CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and Muscle Aryl Hydrocarbon Receptor Nuclear Translocator-Like) that upregulate the second circuit made of genes PERIOD (PER1, PER2) and Cryptochrome (CRY1, CRY2). The latter are expressed, then accumulate, dimerize, and are transported into the nucleus to feedback the core circuit CLOCK and BMAL1 resulting in the repression of transcription of these genes.

The cycle takes about 24 hours. PER and CRY proteins turn over in a tightly regulated fashion mediated by protein phosphorylation with either CK1 (casein kinase 1), or AMPK (AMP kinase), respectively, to end the cycle by protein degradation with E3 ubiquitin ligase complexes.

There is additional feedback control by another loop that involves the induction of two other direct targets of CLOCK-BMAL1, which consists of nuclear receptors (NRs). These are, REV-ERBα and REV-ERBβ (retinoic acid-related orphan nuclear receptors), a transcriptional repressor, and RORα, RORβ and RORγ (RAR-related orphan receptor A isoform 1) that regulates transcription of BMAL1, leading to an “antiphase oscillation in Bmal1 gene expression”. There are several other transcriptional targets of CLOCK-BMAL1, illustrated in Figure 5, which give additional complexity to the feedback loops. Among these are several other nuclear receptors and transcription factors that are members of the PAR-bZip (Basic Leucine Zipper Domain), and bHLH (basic helix-loop-helix proteins) regulated as circadian outputs of rhythmic biological processes [23].

The core oscillators, the heterodimers CLOCK-BMAL1, seem to do their work by promoting the rhythmic removal of nucleosomes at it’s target sites, as well as rhythmic

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**Figure 2: Integration of the three branches of the UPR: cell survival or death.** The upper panel depicts the main functions of PERK, IRE1, and ATF6, for either survival or apoptosis. The lower panel shows the molecular pathways associated to each branch of the UPR, and their respective transcription mediators.
Figure 3: The Rhythm of Life. The diagram depicts the complex array of relationships between the SCN (entrained by light and food), and the downstream events triggered by the release of hypothalamic hormones, and the induction of nuclear receptors and other peripheral clocks. These contribute to synchronize the central clock with energy metabolism and other important processes such as reproduction and blood pressure. The cycle ends by releasing signals that feedback the SCN, to fine-tune a variety of key physiological functions affecting body temperature, blood pressure, glucose homeostasis, feeding, energy outlay, behavior and sleep/wakefulness. Adapted from (121)

Figure 4: The Central Clock. Two major circuits of gene products are responsible for the control of circadian rhythm in mammals by the SCN and peripheral oscillators. At the core, transcriptional activators CLOCK, and BMAL1 upregulate the second circuit made of PERIOD (PER1, PER2) and Cryptochrome (CRY1, CRY2). The latter are expressed to then accumulate, dimerize, and are transported into the nucleus to feedback the core circuit CLOCK and BMAL1 resulting in the repression of these genes. The cycle takes about 24 hours, and PER and CRY proteins turn over in a tightly regulated fashion mediated by protein phosphorylation with either CK1, or AMPK, respectively, to end the cycle by protein degradation with E3 ubiquitin ligase complexes (Proteasome). There is additional feedback control by another loop that involves the induction of two other direct targets of CLOCK-BMAL1 consisting of nuclear receptors (NRs). These are, Rev-Erbα and Rorα that regulate transcription of Bmal1. Blue arrows with (+) signs mean induction and/or activation, and red arrows with (-) signs mean repression of transcription.
modifications of chromatin, with the participation of other transcription factors such as HNF6 (hepatocyte nuclear factors) that are involved in cholesterol, fatty acid, glucose transport and metabolism in many different tissues [124].

The circadian outputs of the SCN, therefore, connect and feedback the central clock to a host of NRs, depicted in Figure 5, comprising a family of 48 transcription factors (in humans). These NRs are involved in the control of a myriad of complex networks of universal and tissue-specific biological processes, such as tissue homeostasis, as well as development, reproduction, energy metabolism and systemic inflammation. Legend: Blue and red arrows are as indicated in Figure 4, solid lines mean direct interactions of NRs with core components of the circadian clock (125, 128).

Other factors that affect the circadian clock include posttranslational modifications, such as acetylation, phosphorylation, ubiquitination, and sumoylation [125]. In particular it is noteworthy the regulation by phosphorylation of REV-ERBα by protein kinase GSK3b [127], since the former, as illustrated in Figure 4, is a key component of the core clock circuit, and an important regulator of lipid metabolism and up to 80% of NRs subjected to circadian rhythms [128].
To add even more complexity to the regulation of NRs by the central clock, there is also posttranscriptional modification affecting the translatability of specific mRNAs, as is the case, for example, of the clock output gene \textit{NOC} (Nocturnin). \textit{NOC}, a member of the deadenylase superfamily, regulates the length of poly (A) tails of mRNAs, and controls the subcellular localization of PPAR-\gamma (Peroxisome proliferator-activated receptor gamma), a hub that regulates the networks of fatty acid storage and glucose metabolism [129]. In response to nutrient status, there is binding of NOC to PPAR-\gamma to induce its translocation to the nucleus, enhancing its transcriptional activity and further promoting adipogenesis [130].

As we will see in the next section, it is through this complex network of NRs, as well as through cellular metabolites, that ER stress connects to the circadian clock oscillators, regulating energy metabolism, systemic chronic inflammation, and the development of NCDs, both upstream and downstream of the UPR.

\section*{7 Crosstalk of the UPR with the oscillators of energy metabolism}

The UPR directly connects with key oscillatory gene products that act as hubs controlling complex networks of energy metabolism, namely, NRs and regulatory enzymes, as illustrated in Figure 6. The master gene of the UPR, \textit{ATF6\alpha}, for instance, upregulates the gene expression of the nuclear receptor ERR\gamma (Estrogen-related receptor gamma) in response to ER stress. Moreover, this transactivation requires co-activator PGC1\alpha, which together with \textit{ATF6\alpha} binds to the corresponding promoter to upregulate ERR\gamma. Additionally, there is also crosstalk between both transcription factors since the latter is also capable of inducing \textit{ATF6\alpha}, again in cooperation with PGC1\alpha (intervening in histone H3 and H4 acetylation). Thus a positive feed-forward loop controls the gene transcription of both \textit{ATF6\alpha} and ERR\gamma [131].

As it is known, ERR\gamma, a member of the nuclear hormone receptor family of steroid hormone receptors, is a downstream mediator of glucagon action in liver gluconeogenesis, and may serve as a suitable target for the treatment of insulin resistance and type 2 diabetes [132]. The cross talk of \textit{ATF6\alpha}, ERR\gamma and PGC1\alpha is interesting because the latter two are subject of circadian behavior by REV-ERB\alpha [128]. Moreover, \textit{ATF6\alpha} itself seems to control the mRNA expression of \textit{PER1}, one of the canonical components of the second circuit of gene oscillators of the SCN, and if that turns out to be the case, then it would constitute an indirect modulation by the UPR of the circadian clock through an \textit{ATF6\alpha}/\textit{PER1} circuit [120].

Downstream of the UPR, a key transcription factor of the PERK branch, \textit{ATF4}, is directly regulated by \textit{CLOCK}...
at the transcription level, and the loss of the former can disrupt the expression of other clock components, including the PER2 gene [133]. A third point of molecular crosstalk of the UPR and the circadian clock is provided by XBPI, the splicing product of branch IRE1α, since its shows a 12-hr peripheral oscillation in the ER of mouse liver that was concurrent with the circadian regulation of BIP expression, a gene target of ATF6. Moreover, in that experimental model, the lack of a circadian clock provoked the deregulation of ER enzymes and compromised lipid metabolism [134]. The implication, is that all branches of the UPR are sensitive and oscillate with a circadian rhythm [120].

There might also be crosstalk between the UPR and both the central and peripheral oscillators through sensors of energy metabolism that synchronize the rhythmic accumulation of metabolites with the activity of the core components, BMAL1 and CLOCK. A fundamental role connecting the circadian rhythm with energy metabolism is carried out by NAD+-dependent deacetylase, SIRT1, an enzyme that in response to cellular levels of NAD+ controls PER2 and BMAL1 in peripheral tissues, as well as the central components BMAL1 and CLOCK of the SCN.

The enzyme SIRT1 activates the transcription of BMAL1 and CLOCK with the involvement of PGC1α and NAMPT (Nicotinamide phosphoribosyltransferase), a biosynthetic enzyme of NAD that ensures the rhythmic accumulation of NAD+ and SIRT1 activity [135]. SIRT1 is also a “hub embedded in a network of hubs” that regulates the activities of a wide range of oscillatory NRs that are important in cellular metabolism, in particular those that regulate lipid, cholesterol and glucose homeostasis such as PPAR-γ and LXR (liver X receptor), as well as the coactivator PGC1α [136]. Thus, it appears that SIRT1 integrates oscillatory metabolic processes sensed by NAD+ that are regulated by NRs and their respective coactivators [137].

It turns out that XBPI is also a target of SIRT1 action, inhibiting the transcriptional activity of the former by deacetylation. Consistent with this fact, under ER stress in mouse embryonic fibroblasts, deficiency of SIRT1 upregulates the target gene expression of XBPI and sensitizes cells to apoptosis [138]. Conversely, overexpression of SIRT1 in the liver reduces ER stress and insulin resistance in genetically obese mice. In addition, the hepatic overexpression of SIRT1 hinders the UPR and improves receptor signaling in the liver, augmenting glucose tolerance and decreasing hepatic gluconeogenesis [139].

The details of how the regulation of XBPI by SIRT1 synchronizes the UPR with the oscillations of energy metabolism are still unresolved. It seems clear, however, that in hepatocytes, the circadian clock does synchronize the rhythmic activation of the IRE1/XBP1 branch of the ER, thus the absence of circadian clock impairs the UPR in those cells [134, 140]. Knowledge of the final details will undoubtedly represent another level of redundancy and feedback with the direct effects on the three branches of the UPR by the core components, BMAL1, CLOCK, as well as by REV-ERB.

8 Crosstalk between the UPR with central and peripheral oscillators of systemic chronic inflammation.

The three branches of the UPR also crosstalk with the oscillatory hubs of systemic inflammation networks, as illustrated in Figure 7. Such interactions comprise the NFkB signal transduction cascade, the JNK-AP1 pathway, and networks that respond to oxidative stress signaling. The relationship of ER stress with inflammation is well documented and better characterized [6], but less is known about the peripheral oscillatory character of some of these hubs [141, 142] and their possible links to the circadian clock [143].

In brief, the NFkB belongs to a family of hetero-dimeric transcription factors [see Figure 7] that regulate networks made of hundreds of genes controlling inflammation, cell division, and apoptosis [144, 145]. Under non-stimulated conditions, such transcription factors are kept sequestered and inactive in the cytoplasm, by binding to inhibitory molecules of the IkB family (inhibitor of KB).

Upon stimulation by a variety of factors including inflammatory cytokines (i.e. TNF), infectious agents, or cellular stress, the IkB molecules are phosphorylated by the kinase complex IKK (IkB protein kinase) and further polyubiquitinated and degraded by the proteasome. The free NFkB dimer can then enter into the nucleus and bind to a specific promoters or enhancer regions, which activates the transcription of a variety of target genes, including IkB, and others involved in the inflammatory response, growth control and protection against apoptosis [146].

Under conditions of sustained ER stress, two of the UPR branches, IRE1 and PERK, can activate the NFkB-IKK pathway by multiple mechanisms [6], as well as by the ATF6 branch [147]. IRE1 directly activates the NFkB-IKK pathway by its binding to the IKK complex promoting the degradation of IkB, and such activation is apparently mediated by IRE1/TNF-associated factor 2 (TRAF2) which forms part of the complex with IKK [148].
PERK is also capable of activating NFκB by reducing the inhibitor IκB, and the resultant translocation of the dimer to the nucleus to induce the proinflammatory gene expression of TNF and interleukin-1 (IL-1) [149]. ATF6 also activates the NFκB-IKK pathway in cultured cells, in response to bacterial cytotoxins that selectively degrade GRP78/Bip, and although the mechanism is still unknown it involves the transient phosphorylation of protein kinase AKT and further activation of the NFκB-IKK pathway [147].

The UPR crosstalk at the molecular level with systemic inflammation can also occur through CREBH (cAMP-responsive element binding protein, hepatocyte specific), a membrane bound transcription factor of the same protein family of ATF6 [75]. Upon ER stress, CREBH, in a similar fashion to ATF6, is cleaved at two sites by proteases of the Golgi, and translocated to the nucleus to activate the transcription of genes encoding C-reactive protein (CRP) and serum amyloid P-component (SAP), to induce an acute inflammatory response. Moreover, CREBH is also activated by a variety of proinflammatory cytokines that activate the UPR in the liver in vivo, such as TNFα, IL1 and IL6 [75].

The dynamics of the crosstalk of the UPR branches with the peripheral oscillator NFκB are not yet characterized, and likewise little is known about the details of their synchronization to the circadian clock, as this is an emergent field. The peripheral oscillations of NFκB, though, occur as a consequence of the negative feedback loop by which the newly synthesized IκB binds to its nuclear inducer, NFκB, translocating the complex back into the cytoplasm to reininitate another cycle of activation [150].

The oscillations are of importance for the regulation of physiological processes pertaining to inflammatory signaling. Moreover, the frequencies of oscillations, as well as their persistence, seem to be key for controlling the patterns of expression of up to 23-target genes of NFκB at the single cell level, or at the population level in response to proinflammatory cytokines such as TNF [151, 152].

We also know that NFκB induces the transcription of anti-apoptotic genes to suppress TNFα promoted apoptosis, and that the circadian clock regulates such signaling pathway [143]. Interestingly enough, in a mouse model system, the core circadian component CLOCK can upregulate transcription of NFκB target genes in the
absence of BMAL1, and that the latter can counteract the CLOCK-dependent induction of NFκB sensitive genes. Moreover, CLOCK itself can be found associated with the p65 subunit of NFκB, and its overexpression follows the increase in the active and posttranslationally modified forms of p65 (phosphorylated and acetylated).

The molecular link of NFκB to the central clock was confirmed in mouse embryonic cells, and in primary hepatocytes from Clock-deficient mice by the fact that in such systems there is a reduced activation of NFκB in response to the proinflammatory stimuli with respect to wild type cells [153].

There is another feedback loop of the NFκB pathway with the circadian clock, as the RelB subunit of NFκB can interact with core component BAML1 in the presence of CLOCK and repress the expression of circadian genes. In addition, such repression is independent of the negative feedback loop of CRY, and there are also deep alterations of circadian gene expression in mutated RelB -/- fibroblasts [154].

Given the strong effect of the three branches of the UPR upon the NFκB pathways of systemic chronic inflammation, and with several networks of energy metabolism, there might be additional points of crosstalk at the molecular level that integrate the three adaptive processes to the circadian clock. Indeed, such crosstalks are none other than the circadian-regulated coactivators of NRs, which also behaves like hubs of their corresponding adaptive networks.

In the previous section, we have seen how one of such key coactivators, PGCα1, an upstream upregulator of lipid and glucose catabolism, is also a connector to the UPR and the circadian clock. PGCα1 is transcriptionally activated by SIRT1, a target repressed by IRE-XBP1, and the enzyme that sensors NAD levels and regulates PER2 and BMAL1 in peripheral tissues, as well as the core circadian components BMAL1 and CLOCK [134, 135].

In addition to the coactivator PGCα1, the enzyme SIRT1 also deacetylates many other transcriptional regulatory proteins, such as NFκB [135]. Coactivator PGCα1 itself is also involved in the inflammatory response to cytokines, and the evidence comes from the fact that overexpression of TNFα in transgenic mice reduced the expression of coactivator PGCα1 with respect to control mice, apparently with the involvement of the NFκB pathway [155].

Another point of crosstalk of the UPR with peripheral oscillators and the circadian clock is in the tumor suppressor p53, a potent responder to genotoxic stress capable of inducing cell-cycle arrest, senescence, or apoptosis. As illustrated in Figure 7, p53 also intervenes in the control of several networks of systemic chronic inflammation, in particular in those pathways directly regulated by NFκB [156].

Tumor suppressor p53 is an antagonist of the UPR [157], an oscillator itself [158, 159], and a regulator of PER2 expression [160]. In p53-deficient mice, submitted to chronic ER stress by tunicamycin (an ER-resident glycosylase), there was a more pronounced UPR (i.e. induction of GRP78/Bip and GRP94) than in control, and in addition, the alternative splicing of XBP1 was more efficient in cells that did not express p53, pointing to an antagonizing role of p53 on the UPR [157].

Conversely, ER stress can also inhibit p53, perhaps as a mechanism to restrict p53 in response to genotoxic stress (i.e. DNA double strand breaks, or stalled DNA replication forks) [161]. In brief, the mechanism by which ER stress inhibits p53, involves the increased localization and degradation in the cytoplasm of p53, by phosphorylation at serine 315 and serine 376 mediated by GSK3β (glycogen synthase kinase 3) in the nucleus. Contrarily, when this enzyme phosphorylates p53 in the cytoplasm, but at serine 15 and serine 20, it causes the translocation of p53 to the nucleus where it exerts its transcriptional effects. Proof of such an effect is provided by experiments with GSK3β -/- cells submitted to ER stress, where p53 remained in the cytosol and did not produce apoptosis upon DNA damage [162].

The dynamics of the oscillatory character of p53 is well documented in vivo using bioluminescent imaging following ionizing radiation [163], as well as at the level of individual living human cells exposed to DNA-damaging gamma radiation. As a model, the experiments used a negative feedback loop involving fluorescently tagged p53 and Mdm2 (murine double minute oncogene), also known as E3 ubiquitin-protein ligase Mdm2, a negative regulator of p53 [158].

The oscillations of p53 were so important that, by use of computational modeling, it was possible to show that recovery of cells from DNA damage was dependent on p53 pulses, whereas sustained signaling of p53 made the cells undergo senescence. Protein dynamics of p53, therefore, might be as important as the signal itself to influence cellular fate decisions [159].

At the SCN level in mouse, p53 also appears to play an important regulatory role since p53 -/- mice displayed a shortened period, and a disrupted circadian control, in response to light stimulation (photo-entrainment to light pulses). The mechanism involves the blocking by p53 of the binding of CLOCK/BMAL1 to the PER2 promoter, resulting in the repression of PER2 expression [160]. Not much is known about how such a direct effect of p53 on the circadian clock of the SCN interconnects with its own pulsating effect on peripheral tissues.
It is clear, however, that biological oscillators resulting from feedback loops of key regulators or hubs, are responsible for the interconnectivity and synchronization of cellular stress pathways to the SCN, including those of the UPR and other networks of adaptive responses that control cell fate.

9 Conclusions and implications regarding the NCDs, of the UPR integration to other adaptive processes linked to the circadian clock.

We approached this review to explore as a whole, the many interactions of adaptive systems of systemic chronic inflammation, ER stress, and energy metabolism regarding the NCDs, that may help us to get at least some partial answers to the following two fundamental questions:

1. How dependent are the adaptive responses and subsequent events leading to NCDs to their state of synchrony with the SCN and peripheral oscillators? And,

2. How could modifiers of the activity of the SCN clock, for instance, food intake, exercise, and drugs, be potentially used to modulate systemic inflammation and ER stress to ameliorate or even prevent NCDs?

The exact answer to the first question remains unsettled. Much of the evidence reviewed here on the interconnectivity of the UPR with peripheral oscillators of energy metabolism, systemic chronic inflammation, and cell proliferation with the central clock, point to a strong affirmative response. The UPR must be highly dependent on its capacity to integrate and synchronize with the central and peripheral oscillators regulating cell fate (survival, senescence, apoptosis, and necrosis). On this matter we have reviewed the effects of two key regulators of cell fate directly connected to the UPR, the p53 pulses on cell repair [159], as well as the oscillatory NFκB regulating systemic inflammation [164].

We have also discussed results of many papers, which reveal several points of crosstalk of the UPR with key oscillatory proteins regulating transcription hubs, in particular the NRs. In this regard it was particularly interesting to observe the positive feedback loop of the master regulator ATF6, with the transcriptional activation of an important regulator of liver gluconeogenesis, ERRγ. This process requires the coactivator PGC1α, which is also a molecular switch to many metabolic pathways and a regulator of the peripheral circadian clock [131, 165]. Coactivator PGC1α regulates energy metabolism and is expressed rhythmically in the liver and skeletal muscle of mice, and controls circadian rhythm by stimulating the expression of BMAL1 and REV-ERBα by coactivating of ROR receptors [126].

The interconnections of ATF6 with the circadian clock may even go farther than the circuit with ERRSγ-PGC1α, given the apparent modulation by the former of PER1 transcription, a core component of the negative feedback loop repressing CLOCK-BMAL1 [120]. The interdependence of the UPR branches to the core components of the circadian clock is further strengthened by the ATF4 upregulation of PER2 gene transcription in an oscillatory manner in cultured fibroblasts, as well as in the SCN [133]. Moreover, in human cancer cell lines, ATF4 is transcriptionally modulated by direct binding of CLOCK to an E-box element located in the promoter of ATF4 [166].

Another point of interconnection and synchronization of the UPR with the circadian clock was provided by the crosstalk of ATF6 and IRE1α in mouse liver cells, resulting in a 12hr oscillatory cycle of activated XBP1, a rhythm that could be disrupted by the absence of the circadian clock, producing serious perturbations of the ER-localized enzymes of lipid metabolism [134].

Therefore, the partial answer to the first question is that there is firm evidence to document the close interdependence of the three branches of the UPR, with the core components of the circadian clock, as well as with peripheral oscillators of other adaptive networks of cell fate such as those of systemic inflammation, cell proliferation, apoptosis, and necrosis [120]. The exact details of such molecular interactions, and most importantly, their corresponding dynamics, remains to be resolved but this emerging field of biological oscillators will give abundant answers in the foreseeable future.

The second question is how could modifiers of the activity of the SCN clock be potentially used to modulate systemic inflammation, ER stress and metabolic changes, and ultimately ameliorate or even prevent NCDs? The answer to this question is even more intricate, but perhaps more enthralling than the answer to the first question posed in this review. Given the strong dependence on the central clock of these adaptive responses to cellular stress, it is then imperative to change the focus of preventative and therapeutic treatments of NCDs.

The new paradigm should not emphasize just the most obvious and reductive risk factors preceding NCDs because those, rather than causes leading to disease, are consequences of disequilibria of the multiple adaptive responses to cellular stress. Among such risk factors,
are obesity, hypertension, metabolic disturbances like hyperglycemia or dyslipidemias (high blood triglycerides, high LDL), and systemic chronic inflammation (i.e., indicated by CRP, cytokines, adiponectin).

The preceding sections showed how close the relationships and feedback networks are for these adaptive responses, as well as the relationship on the central clock and peripheral oscillators. The new paradigm to prevent and treat NCDs, therefore, should pursue as a main objective the highest degree of synchrony of such adaptive networks to cellular stress.

The preventive and curative strategies for NCDs should focus on a systems approach to analyze and correct potential and current disturbances, which may contribute to major sources of uncoupling or asynchrony of the SNC with peripheral oscillators. These major sources can include: time of food consumption, the source of calories and their corresponding impact on the microbiome, physical activity, hormone signaling (e.g. cortisol, insulin), light entrainment, and non-photic zeitgebers including social life and mental state, of both patients and healthy individuals, or at risk to NCDs [122, 167-169].

At the molecular level, as illustrated in Figures 5 and 6, the prime candidates to monitor the state of synchrony of peripheral oscillators with the SCN will be the hubs connecting metabolic networks to the central clock; that is Sirtuins, NRs and nutrient-sensing transcription factors (e.g. CREB, FOXO), coactivator PGC-1, and certain protein kinases such as AMPK, among others [167].

Equally important as preventive and therapeutic targets to major NCDs, are the points of crosstalk of the branches of the UPR with the central clock as illustrated in Figure 7. The same can be said to macro molecular interactions of p53 to recover homeostasis, as well as that of NFkB and the pathways of systemic chronic inflammation, as depicted in Figure 7.

It comes at no surprise then, that DNA polymorphisms of most of these hubs, including those of the central clock, have predictive value for NCDs. This is the case of an increased risk of cancers associated with variants of BMAL1, CLOCK, CRY1, CRY2, PER3, and of Casein Kinase 1ε [120]. The same can be said for polymorphisms of the genes of ATF6 [170], XBP1 [171], NFkB [172], and, undoubtedly, of variants of p53 [173]. Regarding increased risk to lipid metabolic disorders, diabetes, and cardiovascular disease, there is also predictive value in variants of SIRTUIN1 [174], AMPK [175], and NFkB [176].

The analysis of the synchrony of a whole organism with respect to the central clock may now sound far-fetched and extremely complex or even worst, complicated and not feasible. However, recent advances and lower costs of genomics and proteomics will make this possible, feasible, and cost-effective as it is the case of personalized oncogenomics, and other emerging techniques such as activity-based protein profiling [177, 178].

The cost of ignoring the systems approach to preventive and curative medicine may be enormous, both economically and in human lives. Perhaps the best illustration of this fact is the drug rosiglitazone, a thiazolidinedione employed to treat type 2 diabetes. The drug targets the hub PPARγ, a highly connected NR, but it was banned in Europe because of its serious side effects, such as congestive heart failure, myocardial infarction, and death in clinical settings [179].

More recently, and by using a systems approach, a recent report uncovered that rosiglitazone does increase atherosclerosis and induces cardiac hypertrophy. The mechanism seems to obey an alteration in cardiac energy metabolism by down regulating two metabolic hubs, coactivator PGC1α and PPARα. The metabolic shift caused its deleterious effects through decreased fatty acid oxidation and increased glucose utilization [180].

In summary, our heuristic inquiry led us to trace gene hubs, network modules and feedback loops of crosstalk of the UPR, with different adaptive responses, from the level of the entire organism down to the intracellular molecular milieu. From there back up, it was possible to uncover a framework of afferent and efferent connections of the three branches of the UPR, with the corresponding networks of systemic chronic inflammation [181], circadian rhythm, and dysbiosis, as well as with several mechanisms controlling cell fate [182-184]. The outcome of such a synthesis was graphically depicted in figures 6 to 7, and narrated here in Conclusions and Implications.

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