# When Endoplasmic Reticulum Proteostasis Meets the DNA Damage Response

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**Abstract**

Sustaining both proteome and genome integrity requires the integration of a wide range of mechanisms and signaling pathways. The latter comprise, in particular, the unfolded protein response (UPR) and the DNA damage response (DDR). These adaptive mechanisms take place respectively in the endoplasmic reticulum and in the nucleus. Alterations in UPR and DDR are associated with aging and with the occurrence of pathologies such as degenerative diseases, metabolic and inflammatory disorders, and cancer. Here we discuss the emerging signaling crosstalks between the UPR stress sensors and the DDR and their implication in cancer biology.

## Introduction

Maintenance of protein homeostasis (**proteostasis**) is mediated by a network of interconnected quality-control processes, ensuring a functional proteome [1]. Deregulation of endoplasmic reticulum (ER) proteostasis is a common feature of several metabolic, degenerative, immunological, or neoplastic diseases [2,3]. ER proteostasis surveillance is mediated by the **unfolded protein response (UPR),** a signal transduction pathway that senses protein biogenesis defects in the ER [2]. Likewise, alteration of **genome integrity (GI)** and the mechanisms involved in GI maintenance, prevent inherent and sporadic genetic diseases. An evolutionarily conserved mechanism, the **DNA damage response (DDR)** ensures GI through the recognition of DNA lesions, followed by the initiation of a signaling cascade resulting in DNA repair [4]. Recently, failure in maintaining GI was associated with ER proteostasis alteration [5–10]. In addition, some studies now support a fundamental biological function for UPR sensors in the maintenance of GI and DNA-damage gene expression [11–14].

In this review we describe the UPR and DDR sensors, their mechanisms of action, their impact on global proteostasis and on the activation of the DDR. We discuss emerging connections between the UPR and the DDR and we focus on cancer, given the relevance of both pathways as hallmarks of this disease.

## ER Proteostasis and the Unfolded Protein Response

The ER is the gateway to the secretory pathway through which ~30% of the cellular proteins transit. Proteins acquire proper folding and conformation in the ER, thus making this compartment a key contributor to cellular proteostasis [1]. ER proteostasis disruption can be due to i) malfunctions of ER proteostasis control mechanisms, ii) the accumulation of improperly folded proteins, or iii) the imbalance between protein folding capacity and demand, and yields a condition called ER stress.

To cope with ER stress, the UPR, a homeostatic signaling pathway that aims at restoring ER proteostasis, is triggered to increase ER protein folding and clearance capacity or to promote cell death programs if the stress cannot be

resolved (see box 1) [15,16]. Under basal conditions (non stressed), it is believed that ER stress sensors are maintained inactive through the binding to the ER luminal chaperone **binding immunoglobulin protein (BiP)**, whereas the accumulation of misfolded proteins triggers BiP release from the stress sensors thereby allowing their activation (Figure 1) [17]. The execution of the UPR results in i) the reduction of misfolded proteins in the ER resulting from the transient attenuation of mRNA translation; (ii) the improvement of the ER folding capacity by increasing the expression of ER-resident chaperones proteins; (iii) the enhancement of ER protein clearance by increasing its degradation capacity (e.g., through the **ER-associated degradation (ERAD)**[18]); (iv) the enhancement of the export capacity (e.g. through the upregulation of the expression of several genes whose products are involved in ER cargo exit) (Figure 1) [19].

The UPR is transduced by three ER-resident proteins, **IRE1α**, **PERK** and **ATF6α**, whose primary function is to activate the signalling pathways whose aim is to restore ER proteostasis [2]. However, when ER stress cannot be resolved, these UPR sensors activate death signalling pathways (see Box 1) [1]. IRE1α is a type I transmembrane protein, that exhibits kinase and endoribonuclease (RNase) activities in its cytosolic domain. Under ER stress, IRE1α oligomerizes, then trans-autophosphorylates which triggers a conformational change that activates the RNase domain. IRE1α RNase activation, together with the tRNA ligase RtcB, induces the non-conventional splicing of X-box binding protein 1 (XBP1) mRNA, [2,20–22]. The spliced XBP1 mRNA encodes the transcription factor XBP1s which promotes the transcription of a number of genes whose products are involved in the ER proteostasis such as foldases, oxidoreductases and ERAD components (Figure 1) [2,16]. Alternatively, IRE1α RNase degrades multiple mRNAs and miRNAs in a sequence-specific process called regulated IRE1α-dependent decay (RIDD) of RNA [23]. Although RIDD activity has been proposed to be necessary for the maintenance of ER homeostasis [24,25] and the pathogenesis of diabetes [26], cancer [27] and inflammatory conditions [28,29] most of the available evidences are difficult to interpret due to the concomitant activation of *Xbp1* mRNA splicing and RIDD activity.

PERK is a ubiquitously expressed type I transmembrane serine/threonine kinase. Under ER stress, PERK oligomerizes and trans-autophosphorylates to acquire full kinase catalytic activity and to phosphorylate the eukaryotic translation initiator factor-2 (eIF2α), thereby attenuating the general protein synthesis [2,30]. This limits the entry of newly synthesized proteins in the ER while allowing the selective translation of a growing set of specific mRNA such as that coding for the activating transcription factor 4 (ATF4), a transcription factor which promotes the antioxidant response, amino acid metabolism, ER folding capacity, upregulation of macro-autophagy and therefore has an important pro-survival role (Figure 1) [2]. Additionally, ATF4 expression engages the apoptotic program through the expression of CHOP protein (also known as GADD153), a transcription factor that upregulates the pro-apoptotic members of the BCL-2 family and GADD34 (see box 1) [2,31].

ATF6α is a single-pass type II transmembrane protein located in the ER under resting conditions. ATF6α bears a bZIP transcription factor on its cytosolic domain that is released upon ER stress [2,32]. The accumulation of improperly folded proteins in the ER causes ATF6α to be exported to the Golgi apparatus and processed by the S1P and S2P proteases [16]. This process mainly leads to the release of the cytosolic fragment domain of ATF6α [16]. In the nucleus, ATF6α cytosolic domain, simultaneously with XBP1s, upregulates the expression of CHOP and other genes involved in the regulation of ER size, protein-folding capacity, and the ERAD (Figure 1) [33,34].

Remarkably, reprogramming of UPR signaling has been linked with the acquisition of several distinctive hallmarks of cancer [35]. Tumour cells are exposed to several **cell-extrinsic** and **-intrinsic** perturbations that promote the selective pressure to engage the UPR signalling [19,35]. In general, IRE1α and PERK signaling contribute to cancer progression by promoting tumour growth and cell survival in different type of tumours [27,36–39]. However, there are only few studies that link ATF6α activity and cancer. The expression of ATF6α is elevated in colorectal cancer but not in normal mucosa [40] and its expression correlates with a poor prognosis [41]. In human epidermoid carcinoma cells, ATF6α signaling

increases Rheb expression, which in turn activates mTOR signaling (Figure 2) [42]. In addition, protein disulfide isomerase 5 (PDIA5)-dependent activation of ATF6α was described to be instrumental in the acquisition of Imatinib resistance in chronic myeloid leukemia (Figure 2) [43]. Although the role of UPR signaling in the tumour biology is supported by strong evidence [35,39,44], the specific molecular relationship with the genomic instability has not been studied in depth.

**Genome Integrity and the DNA Damage Response**

The preservation of genomic integrity represents a challenge because DNA is constantly exposed to endogenous and exogenous sources of damage. To ensure the genome protection, cells have evolved mechanisms for the detection and repair of DNA lesions called the DDR. The DDR comprises different pathways that can be triggered either by single-strand breaks (SSB) (e.g., mismatch mediated repair (MMR), nucleotide excision repair (NER) or base excision repair (BER)) or by **double-strand breaks (DSB)** (e.g., non-homologous end joining (NHEJ) and homologous recombination (HR)) [45].

DSB are one of the most harmful injuries to the genome [4]. Failure in DSB repair contributes to the genomic instability that drives cancer development [4,46]. The response to DNA double-strand breaks is controlled by three kinases that are members of the phosphoinositide 3-kinase (PI3K)-related kinases family: **ATM**, **ATR,** and **DNA-PKcs** [4]. Those kinases coordinate the phosphorylation of numerous proteins, ultimately regulating a broad spectrum of cellular processes such as DNA replication and repair, cell-cycle progression regulation, and apoptosis or senescence initiation (Figure 1) [47]. Depending on the mechanisms inducing DSB and the cellular context, different kinase pathways are favored such as ATM-Chk2 signaling along with the MRE11-RAD50-NBS1 (MRN) complex [4]. ATR-Chk1 is recruited and activated to DSB by single-stranded DNA coated with replication protein A (RPA) together with its partner ATR-interacting protein (ATRIP) [4]. The MRN complex is recruited to DNA DSB immediately after its occurrence, recruiting ATM to the chromatin and stimulating its kinase activity [4]. ATM activates a widespread DSB-signaling cascade that begins with **H2AX**

**phosphorylation (γH2AX)**. γH2AX is a marker of double-strand DNA damage, its phosphorylation is one of the first response after DSB, and the intensity of the response is proportional to the number and size of DSB foci [48]. Chk2 is activated by ATM and redistributed throughout the nucleus where it functions along with Chk1 in the cell cycle checkpoint signaling network and DNA repair [49]. Chk1 activation after DNA damage is a key function of ATR since activated Chk1 is essential for S and G2/M phase cell cycle regulation [4]. The activation of Chk1 and Chk2 induces the phosphorylation of the transcription factor p53 and the subsequent transcription of p53 target genes [50,51]. These cellular mechanisms are critical in the maintenance of genome integrity and prevention of diseases.

## UPR and DDR signaling in genomic integrity

Maintaining GI is critical to prevent diseases such as cancer, but once cancer occurs it is important to promote the survival of cancer cells. Genotoxic stress is key in most of the cancer treatments, because the collapse of DDR activation and DNA repair mechanisms trigger cell death [52,53]. Interestingly, in patients with a poor prognosis there is a significant correlation between higher BiP expression and chemoresistance[19,39]. Moreover, in a panel of cancer cell lines, genotoxic drugs promote changes in ER structure in a process mediated by the transcriptional activation of p53 (one of the main tumour suppressor and a key player in DDR (see box 1)), which generates the expression of receptor expression-enhancing protein 1 and 2 (REEP1/2) and p53-induced gene 8 (PIG8), three ER-shaping proteins [54].

To date few evidences, connect the UPR and SSB repair. Among them, HMGB1, a protein mainly involved in MMR and BER was shown to be associated with the UPR after ER stress in Huntington's disease. Nonetheless, this link was revealed using a bioinformatic analysis and thus should be carefully considered and confirmed experimentally [55]. In addition, the key BER protein, APEX1, was shown to be induced at the transcriptional level by ER stress in human hepatoma cancer cells [56]. Lastly, ChIP-seq experiments revealed that XBP1s binds to the promoter region of several BER, MMR and NER genes [12,57]. In summary, the

links between UPR and SSB are mainly due to transcriptional control of DDR genes (*BRCA1, FEN1, H2afx, XRCC1, XRCC4, Parp1, Mre11a and Rad51*) by UPR sensors or ER stress stimuli (Figure 2). Aside from this compilation of evidences, most of the links are found to be established upon DSB repair, highlighting the important role of this mechanism in the DDR and UPR proteostasis.

Recently, in human osteosarcoma cells, ATM was shown to differentially regulate proteostasis under DNA damage conditions and **oxidative stress**. Oxidative stress is a natural biological process to which all cells are subjected, and that is recurrent among different pathologies such as degenerative, metabolic, immunological diseases, or cancer [58]. In *C. elegans*, it has been observed that the collapse of proteostasis, associated with the accumulation and aggregation of misfolded proteins, is directly associated with oxidative stress characteristic of aging [59]. Indeed, the expression of mutated forms of ATM resistant to oxidative stress has a slight effect on the DDR, but favors the clearance of toxic protein aggregates [6]. The loss of ATM function under oxidative damage causes wide cellular stress as ATM functions are not limited to its participation in DNA-repair or a specific cellular compartment [6,60]. It is possible to speculate that in physiological conditions, ATM plays a role as an oxidative stress sensor, additionally sensing alterations in other cell compartments, including the ER [10,61]. Accordingly, in *S. cerevisiae*, the ATM/ATR ortholog, Mec1, is a key component of the signaling network promoting survival in response to proteotoxic stress [62]. Mec1 regulates the expression of genes linked to proteostasis, and its inactivation leads to widespread protein aggregation and cell death [62]. Interestingly, protein aggregation is resolved by the activation of autophagy, which facilitates aggregate clearance [62]. Similarly, in mouse fibroblasts, inhibition of chaperone-mediated autophagy leads to hyperphosphorylation and destabilization of the MRN complex and regulated degradation of Chk1 protein [63]. This suggests that autophagy may contribute to GI by ensuring nuclear proteostasis (Figure 1). It has been demonstrated that alterations in the functionality of DDR proteins, e.g.

ATM, correlate with several pathologies other than cancer, such as neurodegenerative syndromes [64,65] or systemic autoimmune diseases [66–68].

## The UPR sensors in the DNA damage response

Several studies have reported functional links between UPR and DDR signaling. Below, we detail the most recent studies in the field, mainly associated with the role of the IRE1α and PERK sensors.

### IRE1α signaling

In *S. cerevisiae*, exogenous expression of mammalian XBP1 was found to play a role in NHEJ DSB repair pathway through the regulation of H4 acetylation [69]. Initially, the regulatory network governed by XBP1s was studied in mouse cells from plasma, pancreatic β, and skeletal myotubes cells, revealing that XBP1s regulates the transcription of a cluster of DNA-repair genes under ER stress [57]. Similar experiments were performed in human hepatic cells confirming that XBP1s directly controls the transcription of multiple DDR genes and the levels of γH2AX (Figure 2) [12]. Moreover, silencing of XBP1s causes an increase in the formation of γH2AX foci as well as a reduction in the expression of MRN complex proteins and in ATM phosphorylation (Figure 2) suggesting an increase in DNA damage coupled with a reduction in damage recognition and processing [70]. XBP1s is not only involved in the regulation of DDR genes but is also directly linked to genotoxic stress response (Figure 2). In human oropharyngeal carcinoma cells, UV-radiation increases the phosphorylation of IRE1α and the expression of XBP1s, thereby triggering an increase in interleukin-6 expression [70]. In addition, in human colorectal cancer cells, exposure to genotoxic drugs, such as doxorubicin and 5-fluorouracil, was found to reduce IRE1α expression and XBP1 mRNA splicing in a p53-dependent manner [71]. However, those results should be taken with caution as the genotoxic stress-induced phenotypes are diverse, and the responses depend on the cell type and agent used. XBP1s regulates the expression of Cul5-ASB11, a ubiquitin ligase targeting BIK, a pro-apoptotic protein [71]. A decreased XBP1s expression reduces Cul5-ASB11 level and increases the expression of BIK

protein under DNA damage. This leads to increased apoptosis, whereas apoptosis is prevented under ER stress [71]. Consequently, ubiquitination and degradation of BIK regulates cell fate in the opposite way, depending on the stress conditions (Figure 2) [71]. It is important to note that in human multiple myeloma cell lines, doxorubicin has been proposed as a pharmacological inhibitor of IRE1α, since it reduces XBP1 mRNA splicing and RIDD activity which in turns leads to a decrease in cell survival [72,73]. This could extend the hypothesis that some genotoxic drugs could also act as pharmacological inhibitors of IRE1α in cells such as multiple myeloma [72], colorectal cancer [71] and triple-negative breast cancer [74].

The activity of UPR stress sensors can be regulated by their binding to co-factors (activators and inhibitors), in addition to post-translational modifications [16]. The concept of the UPRosome emerged to visualize UPR stress sensors as platforms onto which different components assemble to generate composite signals, but also to crosstalk with other signaling pathways to regulate various cellular processes [75]. An example of these multiple interactions is provided by fortilin, a pro-survival molecule that through p53, inhibits ER stress-induced cell death [76]. Fortilin directly interacts with IRE1α, inhibiting its kinase and endoribonuclease domains [76]. Moreover, fortilin silencing increases the expression of XBP1s, which is associated with increased DNA fragmentation and apoptosis *in vivo*. This suggests that the expression of XBP1s increases the signaling of DNA damage, modulating the expression of DNA-repair genes (Figure 2) [57,76].

Recently, a novel IRE1α function associated to the decay of mRNA coding proteins involved in DDR has been identified. In mouse embryonic fibroblasts, genotoxic drugs exclusively engaged the RIDD activity in the absence of XBP1 mRNA splicing [11]. In this model, IRE1α deficiency impairs the ability to repair the genome under DNA damage, disrupting the cell cycle control and the phosphorylation of checkpoint kinases and the histone H2AX [11]. At the molecular level, DNA damage triggers the activation of the c-Abl tyrosine kinase, which operates as a scaffold protein to stabilize IRE1α oligomers and to favor RIDD activity [11]. The role IRE1α activity in controlling the DDR through RIDD was also

validated in fly and mouse models, highlighting a relevant role of this UPR signaling branch in sustaining cell survival and DNA repair under genotoxic stress [11]. In addition, RIDD activity may play protective roles in glioblastoma, and in an *in vitro* assay some DNA-damage proteins could be cleaved by endonucleases domains of IRE1α [27]. These studies highlight the new findings around the RNase activity of IRE1α and its role in pathological conditions, where XBP1s promotes cell death while RIDD enables cell survival [27].

PERK signaling

A direct molecular relationship between PERK and GI is not well documented. However, the activation of PERK-p-eIF2α-ATF4 signaling by single-strand breaks (SSB) was recently described to support cell survival under nutrient-restricted conditions [77]. As the PERK-NRF2 branch contributes to the transcriptional regulation of several genes that mediate the antioxidant response, its alteration has been associated with an increase of ROS after ER stress and the accumulation of oxidative DNA lesions [78]. The downregulation of PERK in human breast cancer cells correlates with an increase in the global phosphorylation of ATM as well as the phosphorylation of its downstream effector Chk2, leading to an increase in γH2AX (Figure 2) [79]. It is interesting to highlight that, as discussed in the previous section, XBP1s downregulation causes an increase in the γH2AX foci but triggers a reduction in ATM phosphorylation [70]. The loss of PERK triggers a significant attenuation of tumour cell proliferation by increased oxidative DNA damage, leading to G2/M cell cycle checkpoint activation [79]. Under ER stress, PERK activation has been shown to operate as a negative regulator of DNA replication in the absence of DNA damage markers by the phosphorylation of the adaptor protein Claspin and the activation of Chk1 [14]. The suppression of the general protein translation by eIF2α phosphorylation reduces cyclin D1 synthesis, generating an impaired activity of cyclin D1-CDK4 complex followed by an inhibition of CDK2, hence ensuring cell cycle arrest at G1 phase (Figure 2) [80]. Under ER stress, PERK activity induces the expression of p47, a truncated p53 isoform, which in turn triggers the upregulation of 14-3-3σ proteins that target the

phosphatase CDC25. This prevents the activation of the cyclin B/CDK1 complex and promotes G2/M arrest, facilitating the ER stress resolution by acting in conjunction with PERK to repress protein synthesis and the ER protein load [81,82]. Interestingly, PERK signaling promotes radio-resistance in human breast and lung cancer cells by increasing the DSB repair signaling [83,84] and chemoresistance in human colon cancer cells by the PERK/NRF2/MRP1 axis [85]. These data open the possibility that PERK inhibitors could be potentially used as a chemo-sensitization treatment [85].

ATF6α Signaling

In a human breast cancer cell model the expression of mutant p53 was shown to enhance the pro-survival activity of ATF6α and to inhibit both IRE1α and PERK branches, damping the activation of CHOP and c-Jun N-terminal kinases (JNK) [13]. This selective activation is necessary for the invasion, migration and cell survival [13]. Furthermore, p53-mutants exhibit increased ATF6α activity (Figure 2) [13]. In addition, ATF6α expression and the engagement of senescence have been described in cells subjected to oncogene activation or to UV-irradiation [86]. Finally, ATF6α expression was shown to contribute to radio-resistance in glioblastoma cells through the upregulation of BiP expression (Figure 2) [87]. Nevertheless, the signaling crosstalk between ATF6α and the DDR and its regulation is not fully characterized, and experimental results are needed to broaden the contribution of this UPR branch to the control, regulation and interaction with the machinery in charge of the genome stability.

Most of the reports available to date focus on the role of IRE1α and PERK as regulators of the transcription of genes coding for DNA damage proteins, which in turn modulate processes such as cell cycle progression and apoptosis engagement impacting the cell fate. The resulting information is pointing towards a direct molecular interconnection between ER proteostasis and DNA damage surveillance as a new and exciting research field where these two homeostatic signaling exert reciprocal and bilateral regulations.

## Concluding Remarks

Recently, several reports have pointed towards interactions between the UPR and the DDR. This suggests a relationship between the ER stress signaling and DNA damage and repair pathways, but the significance of these observations on disease onset is unknown and important questions remain unknown (see Outstanding Questions). Furthermore, new insights about the role of UPR sensors in the maintenance of GI, opens up new perspectives regarding therapeutic targets. Recently, a library of chemotherapeutics compound (> 80 compounds) was shown to induce immunogenic cell death through PERK and IRE1α activation. Future research would be necessary to evaluate more UPR-activation markers in cancer cells [88] after exposure to radiotherapy or chemotherapeutic drugs such as etoposide, doxorubicin, oxaliplatin, paclitaxel or temozolomide; considering the extensive use of these drugs as standard of care in tumour. Moreover, the administration of these drugs is associated with the activation of IRE1α in some models [11,27,72,89,90]. Finally, it is necessary to explore new models to study UPR and the chemotherapeutic response in cancer. Currently, solid tumours represent a suitable model to study the effect of UPR activation in cancer. These types of models have often been useful to reach relevant conclusions about the UPR in cancer, but the inability to correlate with the interaction with extrinsic factors such as metabolic stress, hypoxia, or drug availability will always be a limitation. Furthermore, it would be interesting to study UPR and cancer in suspended or anchor-free cell models. In these models, the current work correlates UPR and its effects on the immune response [91–93], leaving aside the role of chemotherapy or radiotherapy. Thus, the use of anchor-free cancer cells and the generation of UPR sensors-knockout cells eliminates extrinsic factors. However, limitations in anchor-free cell models are related to the expression of surface antigens or the damage-associated molecular patterns (DAMPs) that could be regulated by the UPR under DNA-damage conditions.

The identification of a fundamental biological function for mRNA decay in the maintenance of genome integrity represents a unique example for selective and specific activation of RIDD activity with clear physiological implications.

Remarkably, IRE1α is frequently affected by loss-of-function mutations in various type of cancer [39], contrasting with the notion that cancer cells require IRE1α to survive in hypoxic conditions [35,92]. We speculate that the genetic alterations of IRE1α observed in cancer may synergize with oncogenes to promote genomic instability. Overall, a direct inter-connection is emerging between the pathways that ensure the integrity of the proteome and the genome. It is necessary to explore in depth how unfolded protein response regulates the gene expression, ribosome profile and proteins expression in a context of genotoxic stress, using different multi-omics strategies [94,95], in order to evaluate the global modulation of the DNA damage response. As evidenced in this review, the crosstalk between UPR and DDR is of great interest in the context of UPR biology, especially but not exclusively in cancer biology and treatment.

**BOX1**

**A deadly relationship: Crosstalk between UPR-DDR and their involvement in apoptosis**

In response to unresolved DNA-damage and ER stress the apoptotic program is mainly orchestrated by p53 and CHOP. Both transcription factors are upregulated as a mechanism to monitor the integrity/stability of the genome and proteome. CHOP is upregulated by the UPR arms [96] and it is directly related to the ER stress-induced apoptosis. However, CHOP-deficient cells still undergo apoptosis, suggesting unknown pro-death signaling. CHOP promotes apoptosis through the repression of anti-apoptotic and induction of pro-apoptotic genes, such as, BAD, BIM, NOXA, PUMA and DR5 [97,98]. p53 expression is induced by several stress signals such as DNA damage and oncogene activation [99]. Moreover, p53 has several non-transcriptional functions [100,101]. Senescence, cell-cycle arrest, and apoptosis are the most prominent outcomes of p53 [102]. Principally, p53 engages the apoptosis through the transcriptional regulation of the pro-apoptotic proteins PUMA, BIM, NOXA, and extrinsic apoptotic pathway components [103]. Interestingly, the crosstalk between these two transcription factors has been described. CHOP drives the MDM2 expression, promoting p53 degradation [104]. It has been linked to the function and localization of p53 as a component of the ER stress-induced apoptotic pathway. ER stress promotes p53 expression through NF-κB [105] and CHOP co-operates with FOXO3a to regulate the expression of PUMA and BIM under ER stress [44,106]. Moreover, p53 is an important mediator of ER stress–dependent apoptosis through the upregulation of PUMA [107]. PERK activation modifies the translation of the p53 mRNA from the full-length to the p53ΔN40 (p53/47) isoform and actively suppresses the p21 expression during ER stress, promoting G2 cell-cycle [108]. During chronic ER stress, p53 induces BIK expression while at the same time suppressing BiP translation, leading to the dissociation of the BIK/BiP complex and the apoptosis activation [109]. P53 is located at ER/mitochondria-associated membranes (MAMs) contact sites modulating the $Ca^{2+}$ transfer to the mitochondria [110]. Moreover, p53 regulates autophagy, by the proper localization of PML protein at ER/MAMs [111].

Also, PERK and IRE1α have been identified as components of the ER/MAMs [112,113], suggesting novel interactions between the UPR-sensors and p53. Finally, cancer cells are exposed to several factors that alter proteostasis. To cope with this, tumour cells engage the UPR to manage these disturbances [35]. As p53 mutations are the most recurrent alterations in cancer, leading to the resistance to stressors as DNA damage, the selective inhibition of pro-survival UPR represent a promising intervention on p53-deficient tumours, engaging apoptosis by the induction of unresolved ER stress.

**Glossary:**

**Proteostasis:** Is a network of interconnected quality-control processes in the cell that maintains a functional proteome. Chaperones, foldases, oxidoreductases and glycosylating enzymes ensure that secretory proteins are properly folded, modified and assembled into multi-protein complexes in the ER before they transit further downstream in the secretory pathway.

**Unfolded Protein Response (UPR):** Is a signal transduction pathway that senses the fidelity of protein folding in the ER lumen. The UPR transmits information about protein folding status to the nucleus and cytosol to adjust the protein folding capacity of the cell. The UPR is transduced by three principal ER-resident proteins: inositol-requiring protein 1α **(IRE1α)**, PKR-like ER kinase **(PERK)**, and activating transcription factor 6α **(ATF6α)**.

**Genomic Instability (GI):** Includes all processes that maintain the integrity of DNA such as sensing, signaling and repair of DNA damage, processing of DNA damage in the context of chromatin and chromosomes, cell cycle checkpoint control and apoptosis control. Effective maintenance of genome integrity is essential for healthy organisms, aging, and prevention of diseases.

**DNA damage response (DDR):** Cellular response involving DNA damage recognition, followed by the initiation of a cellular signaling cascade that promotes

DNA repair, which can modulate cell-cycle progression, chromatin structure and transcription, both at sites of DNA damage and globally. DDR after DNA-double strand breaks is controlled by three related kinases: Ataxia- telangiectasia mutated (**ATM),** ATM and Rad3-Related **(ATR)** and DNA-dependent protein kinase **(DNA-PKcs)**.

**Binding immunoglobulin protein (BiP):** Is a key ER chaperone and master regulator of ER functions under ER stress. Detection of misfolded protein species by the three UPR sensors is partly dependent on BiP.

**ER-associated degradation (ERAD):** Is the principal quality-control mechanism responsible for targeting misfolded ER proteins for cytosolic degradation. ERAD targets are destroyed by the cytoplasmic ubiquitin–proteasome system. Many ER chaperones participate into the ERAD complex, including BiP, EDEM1, OS9, and XTP3B. The UPR sensor IRE1α and SEL1L- HRD1 complexes are the two most conserved branches of ER quality-control mechanisms.

**Cell-extrinsic factor:** Any factor that is independent of the genetic background or alteration of DNA, such as hypoxia, glucose deprivation, or inadequate amino acid supplies.

**Cell-intrinsic factor:** Any factor that is dependent on the genetic background or DNA, such as oncogenic activation, alteration in chromosome number or hyperploid.

**DNA double-strand breaks (DSB):** Different classes of DNA damage such as ultraviolet (UV) light, irradiation, DNA-damage drugs or oxidative stress that leads to DNA rupture in both strands. If DNA is not repaired correctly, DSB can cause deletions, translocations, and fusions of the DNA.

**Gamma-H2AX (γH2AX):** Upon DSB induction, the histone variant H2AX is phosphorylated on serine 139 by ATM, ATR or DNA-PK, generating phosphorylated H2AX, or so-called γH2AX. γH2AX induction is one of the earliest events detected in cells and human biopsies following exposure to DNA damaging agents. γH2AX is a key marker of double-strand DNA damages, allowing the activation and relocalization of repair proteins to DSB sites as well as the signal amplification.

**Oxidative stress:** Is an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Amino acids such as proline, arginine, lysine and threonine are particularly vulnerable to oxidative damage, both as free molecules or within proteins. Moreover, oxidative damage can also affect the integrity and stability of DNA and RNA.

**Figure Legend.**

**Figure 1. Unfolded Protein Response and DNA Damage Sensors.** All three ER stress sensors (PERK, IRE1α, ATF6) are localized at the ER membrane and under ER stress they activate signaling events that increase protein-folding capacity and reduce protein load on the ER. In response to DNA damage, ATM is activated and recruited to DSBs by the MRE11-RAD50-NBS1 complex and ATR is recruited to RPA-coated ssDNA by its binding partner ATRIP. DNA-PKcs, meanwhile, is recruited and activated by Ku-bound DSB ends. The UPR transcription factors and DNA damage proteins determine cell fate by the regulation of distinct subsets of target genes spanning from the recovery of ER homeostasis to DNA damage response. The green boxes illustrate the common target and functions induced by the ER stress and genotoxic stress. The blue boxes illustrate UPR functions induced by the ER stress response. The grey box illustrates the DDR proteins involved in the UPR. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

**Figure 2: Unfolded Protein Response sensors involved in the DNA Damage Response.** IRE1α is maintained in a repressed state through an association with BiP. Upon ER or genotoxic stress, misfolded proteins dock to BiP, thus triggering the dissociation from IRE1α. This triggers IRE1α dimerization and auto-transphosphorylation inducing its activation. IRE1α may also bind misfolded proteins to oligomerize. IRE1α allows the splicing of XBP1 mRNA and the degradation of RNAs (RIDD). XBP1s transcriptional factor governs notably the expression of genes involved in DDR and ubiquitin ligases. RIDD activity governs the expression of mRNA, which impact on DDR proteins. IRE1α activity can be modulated by fortilin, c-Abl, p53, doxorubicin or 5-fluorouracil. Knockdown of XBP1 reduces ATM phosphorylation, MRN complex expression and increases γH2AX. The mechanism of PERK repression and activation is the same as for IRE1α. Activated PERK phosphorylates eIF2α, which in turn shuts down global translation and concomitantly increases the expression of the transcription factor ATF4.The

stopping of global translation impact on Rad51, p47 and p53 along with inhibiting cyclin D1 expression, ultimately dysregulating G1 and G2/M cell cycle phases. ATF4 transcriptional factor bind CHOP itself inducing GADD34 transcription that creates a feedback mechanism. PERK silencing increases p-ATM thus triggering γH2AX and Chk2 activation, impacting on cell cycle. ATF6 is exported from the ER to the Golgi where it is cleaved by S1P and S2P proteases, allowing the release of its cytosolic domain, which is a potent transcription factor named ATF6f. PDIA5 and mutant-p53 increase ATF6 activity, promoting Imatinib resistance in cancer and allowing RHEB expression and mTOR signaling. Ub: ubiquitination; P: phosphorylation. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

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## Highlights

- Alteration in the genome integrity has been associated with disruption of the endoplasmic reticulum (ER) proteostasis

- The unfolded protein response (UPR) and the DNA damage response (DDR) play important roles in the development and progression of several diseases, including cancer.

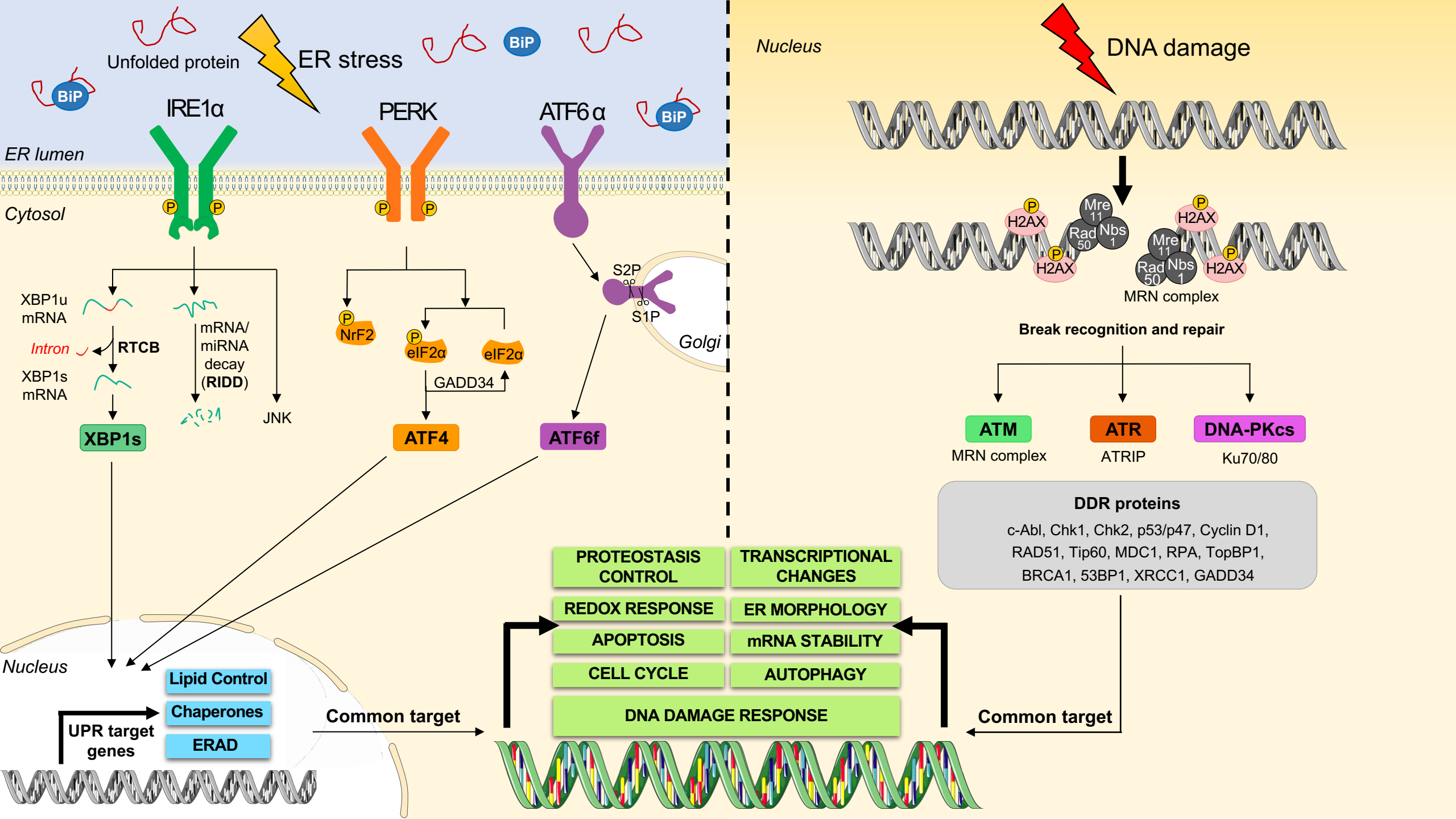
- UPR sensors IRE1α, PERK and ATF6α play a role in response to genotoxic and ER stress in cells by interacting with DNA damage proteins functions (e.g. ATM, ATR, p53, p21, Chk1 or Chk2).

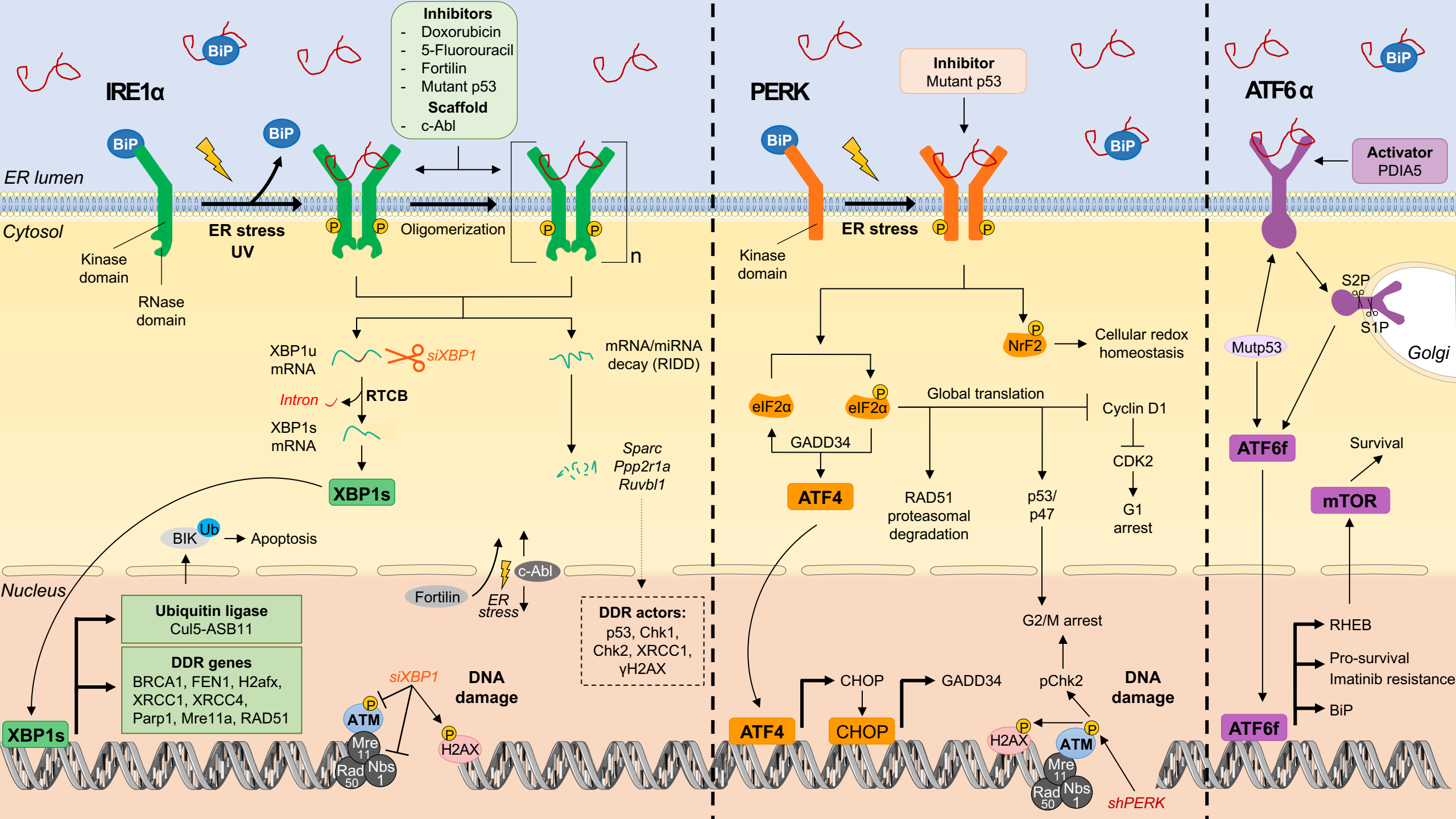
- The crosstalk between UPR and DDR may contribute to cancer progression. Indeed, CHOP and p53 play a central role in the crosstalk between UPR and DDR.

- The pharmacologic modulation of the UPR could enhance the chemotherapy and radiotherapy effectiveness

Outstanding Questions

- Do DDR associated proteins participate in the UPR signaling and regulation?
- Could the UPR impact the chemotherapy or radiotherapy-induced genotoxic stress and therefore modulate the response to cancer treatment?
- Post-translational modifications such as ubiquitination are critical in DDR signaling. Can UPR interfere with DDR proteins stability by modulating post-translational modifications?
- What are the most relevant cancer models that can be used to study UPR and its effect on DNA damage response?


Unfolded protein
BiP
ER stress
IRE1α
PERK
ATF6 α
ER lumen
Cytosol
XBP1u mRNA
Intron
RTCB
XBP1s mRNA
mRNA/ miRNA decay (RIDD)
JNK
XBP1s
NrF2
eIF2α
GADD34
ATF4
S2P
S1P
Golgi
ATF6f
Nucleus
Lipid Control
Chaperones
ERAD
UPR target genes
Common target
PROTEOSTASIS CONTROL
TRANSCRIPTIONAL CHANGES
REDOX RESPONSE
ER MORPHOLOGY
APOPTOSIS
mRNA STABILITY
CELL CYCLE
AUTOPHAGY
DNA DAMAGE RESPONSE
Nucleus
DNA damage
H2AX
Mre 11
Rad 50
Nbs 1
MRN complex
Break recognition and repair
ATM
MRN complex
ATR
ATRIP
DNA-PKcs
Ku70/80
DDR proteins
c-Abl, Chk1, Chk2, p53/p47, Cyclin D1,
RAD51, Tip60, MDC1, RPA, TopBP1,
BRCA1, 53BP1, XRCC1, GADD34
Common target


IRE1α
PERK
ATF6 α
BiP
Inhibitors
- Doxorubicin
- 5-Fluorouracil
- Fortilin
- Mutant p53
Scaffold
- c-Abl
Inhibitor
Mutant p53
Activator
PDIA5
ER lumen
Cytosol
Kinase domain
RNase domain
ER stress
UV
Oligomerization
n
XBP1u mRNA
siXBP1
Intron
RTCB
XBP1s mRNA
XBP1s
mRNA/miRNA decay (RIDD)
Sparc
Ppp2r1a
Ruvbl1
NrF2
Cellular redox homeostasis
eIF2α
GADD34
ATF4
Global translation
Cyclin D1
CDK2
G1 arrest
RAD51 proteasomal degradation
p53/ p47
G2/M arrest
Mutp53
S2P
S1P
Golgi
ATF6f
Survival
mTOR
RHEB
Pro-survival Imatinib resistance
BiP
BIK
Ub
Apoptosis
Nucleus
Fortilin
c-Abl
ER stress
Ubiquitin ligase
Cul5-ASB11
DDR genes
BRCA1, FEN1, H2afx, XRCC1, XRCC4, Parp1, Mre11a, RAD51
DDR actors:
p53, Chk1, Chk2, XRCC1, γH2AX
siXBP1
ATM
Mre 11
Rad 50
Nbs 1
H2AX
DNA damage
CHOP
GADD34
pChk2
shPERK