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Review article

The molecular mechanism and functional diversity of UPR signaling sensor IRE1

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ABSTRACT

The endoplasmic reticulum is primarily responsible for protein folding and maturation. However, the organelle is subject to varied stress conditions from time to time, which lead to the activation of a signaling program known as the Unfolded Protein Response (UPR) pathway. This pathway, upon sensing any disturbance in the protein-folding milieu sends signals to the nucleus and cytoplasm in order to restore homeostasis. One of the prime UPR signaling sensors is Inositol-requiring enzyme 1 (IRE1); an ER membrane embedded protein with dual enzyme activities, kinase and endoribonuclease. The ribonuclease activity of IRE1 results in Xbp1 splicing in mammals or Hac1 splicing in yeast. However, IRE1 can switch its substrate specificity to the mRNAs that are co-transnationally transported to the ER, a phenomenon known as Regulated IRE1 Dependent Decay (RIDD). IRE1 is also reported to act as a principal molecule that coordinates with other proteins and signaling pathways, which in turn might be responsible for its regulation. The current review highlights studies on IRE1 explaining the structural features and molecular mechanism behind its ribonuclease outputs. The emphasis is also laid on the molecular effectors, which directly or indirectly interact with IRE1 to either modulate its function or connect it to other pathways. This is important in understanding the functional pleiotropy of IRE1, by which it can switch its activity from pro-survival to pro-apoptotic, thus determining the fate of cells.

1. Introduction

Proteins are synthesized as linear amino acid chains that need to be folded in a three-dimensional geometry to carry out their biological functions. A specialized cell chamber known as the endoplasmic reticulum (ER) is indispensable for this job. The ER provides the proper microenvironment and the necessary tools to accurately fold proteins [1]. Owing to varied physiological and pathophysiological conditions, the protein folding capacity of the ER gets compromised, leading to ER stress. In order to combat ER stress, cells have developed an ER to nucleus transcriptional signaling pathway known as the Unfolded Protein Response (UPR). The UPR was first discovered in yeast where a single UPR sensor IRE1 is responsible for combating ER stress conditions [2]. In mammalian cells, the UPR is more complex and is mediated by three main signaling branches: IRE1 (Inositol requiring enzyme 1 signaling branch), PERK (PKR-like ER kinase) and ATF6 (Activating transcription factor 6) [3,4]. The net initial effect of UPR is to alleviate ER stress by increasing the amount of molecular chaperones, ER luminal space, and other folding catalysts. However, if the UPR fails to restore homeostasis, it initiates apoptosis [5].

The IRE1 branch of the UPR signaling network represents a highly conserved pathway among the three branches and provides a major platform for deciding cell fate during ER stress [120]. IRE1 is a type I ER transmembrane protein, which assembles into a macromolecular complex that emerges as an independent subdomain [6,7]. The protein possesses dual enzyme activity, kinase and endoribonuclease. Upon activation, IRE1 catalyzes the non-canonical splicing of (Hac1) mRNA in yeast and Xbp1 mRNA in humans [8–10]. In addition, IRE1 degrades a subset of mRNAs localized to the ER leading to their decay through a pathway termed Regulated IRE1 Dependent Decay (RIDD) (Fig. 1) [11,12]. IRE1 ribonuclease activity thus has two different outputs [13]. These two outputs of IRE1 nuclease activity can be activated

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differentially [11], suggesting an unanticipated complexity in UPR. Moreover, Xbp1 and RIDD exhibit different biological outcomes [14]. The IRE-Xbp1 branch has emerged as an important pathway in many human diseases, such as metabolic disorders related to both glucose and lipid metabolism [15]. Notably, IRE1-Xbp1 plays an important role in tumorogenesis, metastatic progression, and chemo-resistance [16]. A recent report showed that Adenovirus (AdV) specifically activates the IRE1-Xbp1 pathway, which in turn boosts lytic infection through a transcriptional feed-forward loop [17]. The role IRE1-RIDD branch in pancreatic β cell has been well established wherein ER stress induction causes RIDD driven degradation of insulin mRNA and pancreatic cell death via activation of NLRP3 inflammasome [15]. RIDD pathway has also been linked with the activation of NF-kB and interferon pathways through RIG-1 (retinoic acid-inducible gene 1) [18]. For a better understanding of the role of IRE1 in cellular physiology/pathology, it is pivotal to characterize the molecular mechanisms engaged in reciprocal Xbp1 splicing decrease and RIDD increase during unmitigated ER stress conditions. Therefore, better understanding of how IRE1 switches between these two ribonuclease activities would have important implications for the medical sciences.

This review brings forward the current research on the molecular mechanism of IRE1 and its modes of action, resulting in its diverse functional role in cells. Through exhibition of structural designs present in both yeast and mammals, we underlined the diversity in the modes of IRE1 activation. This review also covers a detailed account of the molecular mechanism of two divergent ribonuclease activities of IRE1, including Xbp1 splicing and RIDD. So far, it has been established that IRE1's dual ribonuclease activity is of functional significance in cells; therefore, we delineated the latest studies and plausible theories explaining the molecular mediators and mechanisms operating behind

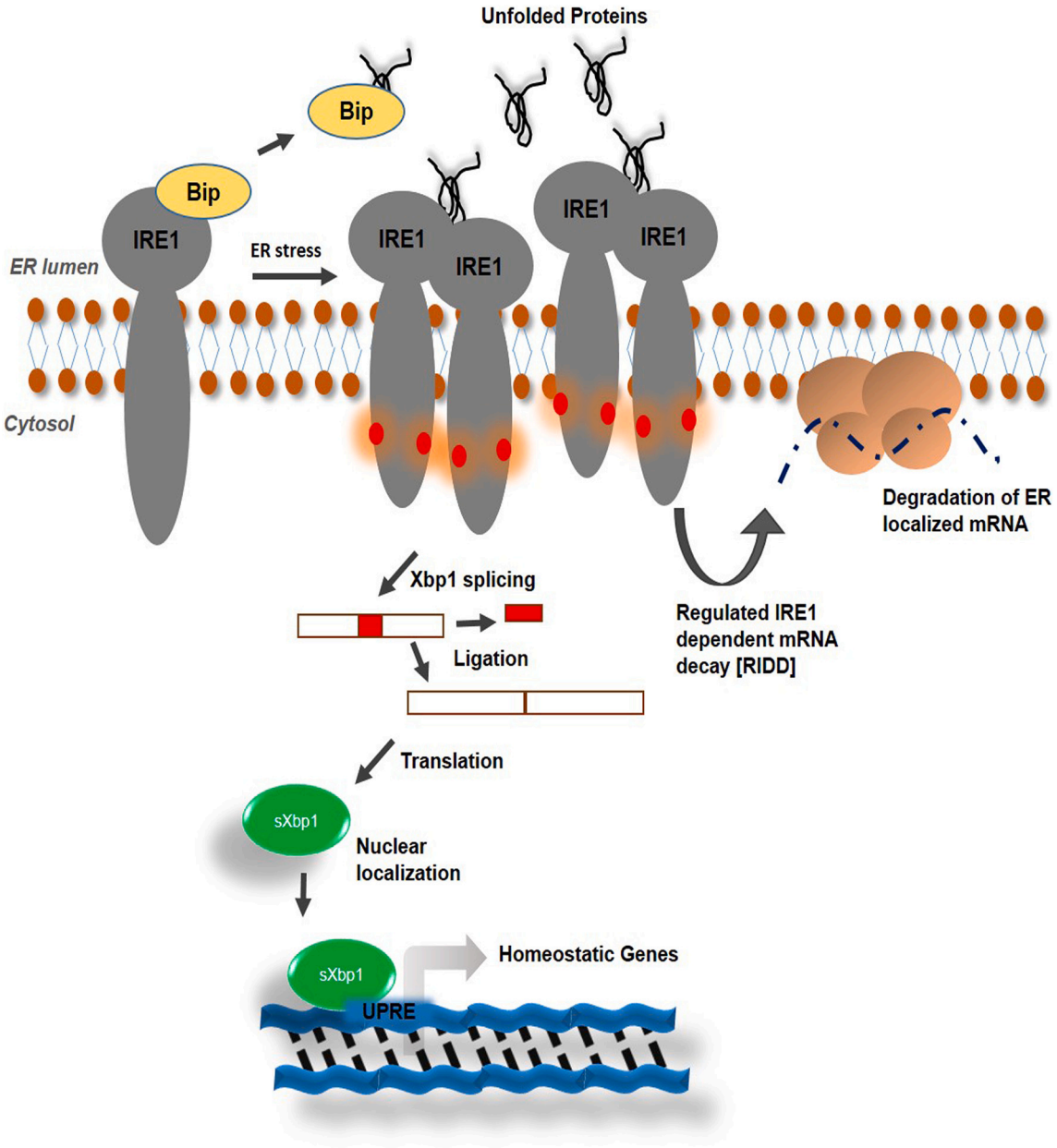

Fig. 1. IRE1 signaling pathway. IRE1 is maintained in an inactive state by Bip binding. Dissociation of Bip upon the accumulation of unfolded proteins leads to IRE1 activation. Activated IRE1 performs the splicing of Xbp1 mRNA that results in the generation of a functional transcriptional factor. Xbp1 translocates to the nucleus, where it binds to UPRE (unfolded protein response element), and drives the expression of genes responsible for ER homeostasis; for example, molecular chaperons, lipid biosynthesis genes, ERAD factors, etc. IRE1 also degrades mRNAs localized to the ER through RIDD.

these functions. The prime importance of the dual ribonuclease activity of IRE1 is that it acts as a switch determining cell fate. We focus on elucidating how different cellular factors communicate and synchronize to switch IRE1 from homeostatic molecular mediator to an apoptotic executor while regulating each of its ribonuclease activities.

2. IRE1: an ER stress sensor and executor

IRE1 acts as a primary sensor of the UPR signaling pathway, and derives its significance from being evolutionary conserved [120]. Sensing of unfolded proteins inside the ER is an important and primary step for a cascade of events that subsequently lead to the activation of the UPR [19]. The IRE1 protein has a peculiar structure and function, which is important in terms of its activation [20–22]. Although the structure and overall function of IRE1 might be conserved from yeast to humans, the difference lies in the execution of downstream signaling and slight structural variation that is responsible for diverse modes of activation present in these two organisms [20,22,23].

2.1. Discovery of IRE1 as a UPR signaling molecule

The mystery of sensing protein folding stress in the ER was solved only three decades ago, when Mary-Jane Gething and Joseph Sambrook; and Mark Rose independently, reported that in yeast, *Saccharomyces cerevisiae* there exists a dynamic pathway regulating protein folding homeostasis – a transcriptional induction program coupled with intracellular signaling from the ER to the nucleus [24,25]. Genetic screens led to the discovery of IRE1 as a main component of UPR signaling [26,27]. Among the molecular mediators of UPR, IRE1 is the only identified ER stress sensor in yeast and is essential for UPR in animals and plants. Because of its conservation in yeast, the molecular regulation of the IRE1 signaling branch has been best studied in that system. Work from two other groups identified the mammalian homolog of IRE1 [28,29]. In contrast to yeast, mammalian IRE1 has two isoforms: IRE1α and IRE1β. The expression of IRE1α is ubiquitous and independent of the cell type, while IRE1β is expressed only in intestinal epithelia. Also, it was found that knockout of IRE1β showed no lethality, however a mouse model displayed embryonic lethality after depleting IRE1α [30,172]. The gene that codes for mammalian IRE1α protein is designated as Ern1 (endoplasmic reticulum to nucleus signaling 1). IRE1 protein is embedded in the ER membrane and screens ER stress through its luminal domain. It executes the kinase and ribonuclease activities through its cytosolic domain [31]. In yeast, IRE1 acts in a linear pathway with Hac1, a transcription factor, as the immediate downstream effector. The transcriptional targets of Hac1 form the set of genes whose major function is to expand the ER and ameliorate stress [32]. However, in mammals, the IRE1 downstream pathway is dissected into two effector functions that either involves the unconventional splicing of transcription factor Xbp1 mRNA or the degradation of a certain set of mRNAs via RIDD [11].

2.2. IRE1 structure and models of activation

The structure of the IRE1 can be divided into the following components: an N-terminal luminal domain, a single-pass transmembrane spanning segment, and a cytosolic region subdivided into a Ser/Thr protein kinase domain and C-terminal endoribonuclease (RNase)

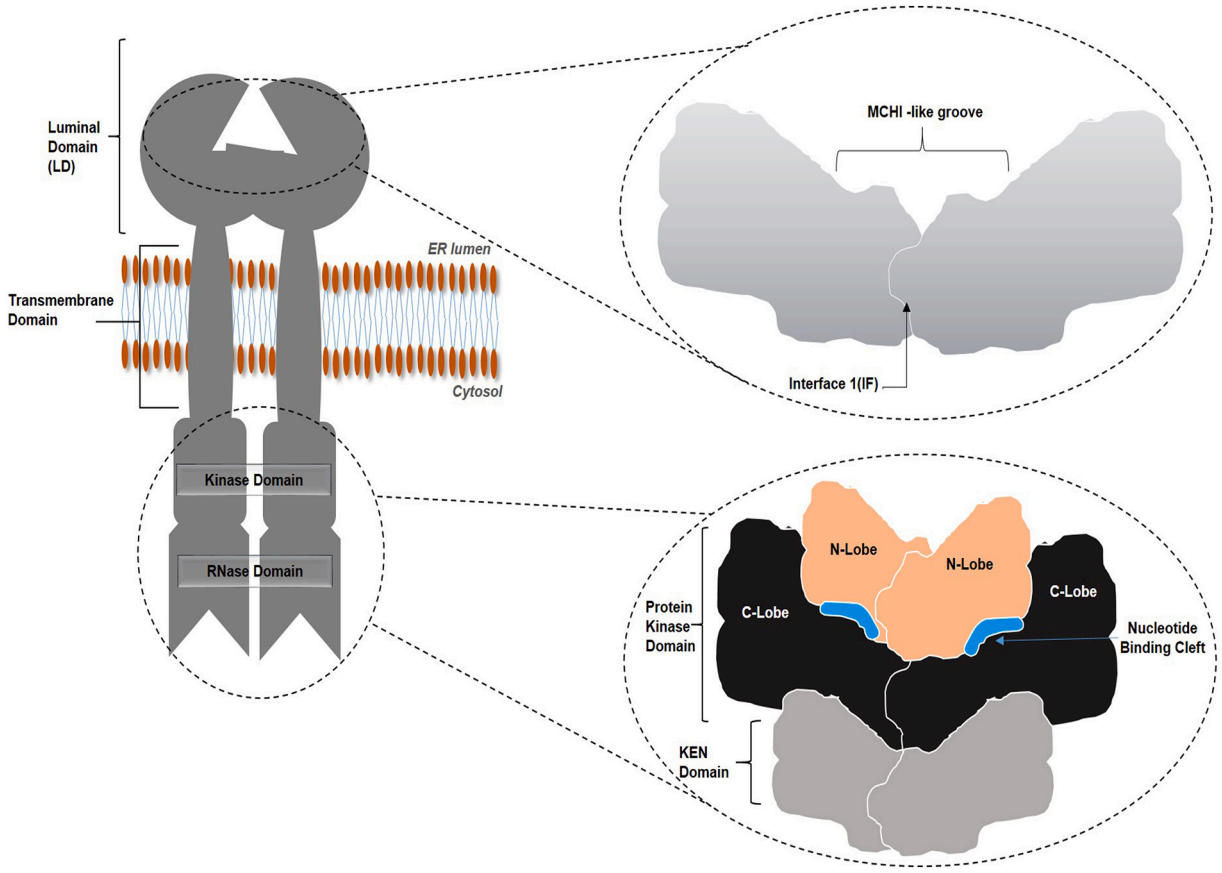

Fig. 2. Structure of IRE1. Structurally IRE1 is divided into three regions: the lumenal domain localized towards the ER lumen, a transmembrane domain embedded into the ER membrane, and the cytoplasmic domain towards the cytoplasm. The lumenal domain of IRE1 packs into a compact structure with the formation of two interfaces; Interface I (IF1) and Interface 2 (IF2). The lumenal domain is highlighted by a deep central groove across IF1. The Cytoplasmic domain of IRE1 is characterized by the presence of two sub-domain, Protein Kinase domain, and KEN domain. The Protein Kinase domain folds in a bi-lobal structure containing N-lobe, C-lobe, and a nucleotide-binding cleft. KEN domain forms a tri-lobal structure by interacting with the C-lobe of the Protein Kinase domain.

domain (Fig. 2). [28]. The detailed insight into the structure of IRE1 and its functional aspects, especially the sensing of unfolded proteins, was revealed by crystallographic and systematic mutational analysis. The mammalian and yeast IRE1 proteins fundamentally follow the same mechanism of activation and downstream signaling, but ER stress sensing in the two organisms follow distinct mechanisms. In yeast, one model suggests that BiP, a predominant ER-resident Hsp70 chaperone associated with inactive IRE1, upon ER stress preferentially binds to accumulating unfolded proteins, thus releasing the inhibitory interaction and liberating IRE1 for oligomerization [33]. But, release of BiP alone cannot be the primary activation step because an IRE1 mutant with impaired BiP binding is still able to respond to the ER stress [20]. The crystal structure studies revealed direct binding model, where the conserved core of the IRE1 luminal domain (LD) directly binds to the unfolded proteins leading to its oligomerization. It was found that LD contains two interfaces; Interface 1 (IF1) creates a two-fold symmetric dimer containing a deep groove, while Interface 2 (IF2) permits further oligomerization. However, there was diminished oligomerization and a decrease in Hac1 mRNA splicing if either of the interfaces was mutated [20]. The groove formed across IF1, constitutes the core stress-sensing region (CSSR) of the dimer that has architectural symmetry with MHC-I (Major Histocompatibility Complex-I) (Fig. 2). The idea evolving from these studies was that in addition to regulation by BiP, this groove in the LD directly senses the unfolded proteins and that binding increases oligomerization [20,21]. Further studies suggested a two-step model for IRE1 activation, in which Bip release from IRE1 leads to IRE1 oligomerization, which is followed by the putative interaction of unfolded proteins with its MHC-I-like groove to trigger full activation (Fig. 3a) [34]. In fact, a remarkable in vivo study carried out in yeast validated this model [35]. Besides the two-step activation model, another study carried out on yeast IRE1 showed that an unknown event, which might be either conformational changes on the luminal side or the transition of IRE1 from dimer to the multimeric form, actually facilitates downstream events on the cytosolic side [36].

Although the sequence of IRE1-LD is conserved from yeast to mammals, including the additional metazoan ER-stress sensor PERK, the difference lies in the sensing of unfolded proteins. Based on the crystal structure, a different model of unfolded protein sensing by human IRE1 sensor domain came forward. The structure suggested that just like the yeast IRE1, the sensor domain of the human IRE1 forms a dimer but not the oligomer unlike yeast. However, the sensor domain dimers are indeed packed into the lattice where the joining is mediated by the smaller crystal-packing interface (dASA = −1182 and −440°A2), whose biological function is yet to be elucidated. As deduced by the crystal structure, the groove formed is narrow and is obstructed in the center by two interacting glutamine side chains, and the flanking helices that are too close to allow binding of the unfolded proteins [22]. This was further supported by the earlier data that unfolded proteins do not bind to the sensor domain but instead are proposed to bind to and sequester BiP, which along with ERdj4 is associated with IRE1 under non-stress conditions [37,173]. Together these studies envisage that high levels of unfolded proteins inside the ER promote the dissociation of BiP from the luminal domain of IRE1, which is enough to induce dimerization and activation of human IRE1 (Fig. 3b) [22,23,37]. Despite the evidences

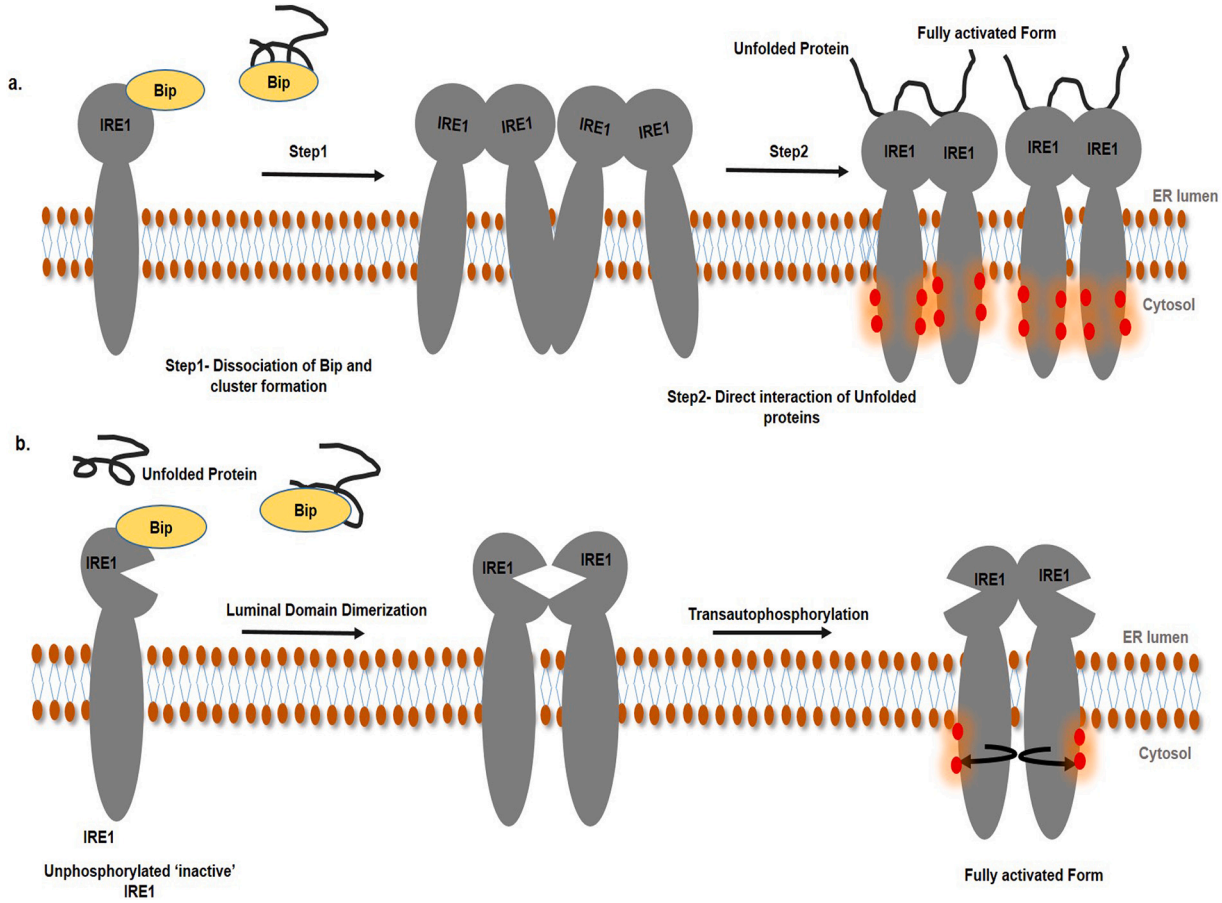

Fig. 3. Activation models for IRE1. a) IRE1 activation in yeast follows a two-step model. The first step involves the dissociation of Bip, exposing the IRE1 luminal domain in a way that promotes cluster formation. This is followed by step 2, where the conformational reorientation is induced by the direct binding of unfolded proteins, which brings the IRE1 to a fully activated state. b) The activation of human IRE1 is underlined by a different model accounting to the differences in the crystal structure. According to this model, IRE1 is positioned in a face to face orientation after the release of Bip from the luminal domain. This position juxtaposes the catalytic centres of IRE1 to initiate trans-autophosphorylation. This brings the conformational change placing IRE1 in a back-back catalytically active state.

supporting a different model for IRE1 activation in mammals, there are a number of studies that redefine this dogma. For example, human IRE1 also assembles into a higher order oligomeric structures through dynamic clustering similar to its yeast counterpart [6,38]. Another study put forth that the binding of misfolded proteins to IRE1 might be involved in inducing allosteric changes that trigger its oligomerization [39]. Furthermore, a quantitative microscopic analysis revealed that the nature of IRE1 oligomers is more intricate rather than simply packed into a lattice. There is a diffusionally constrained core of IRE1 oligomers, but the momomers diffuse and exchange freely with the IRE1 pool present in the ER, although not in a liquid-liquid phase separation manner [6].

The crystal structure studies performed independently on yeast and human IRE1 revealed varied structural elements and rearrangements for downstream signaling subsequent to RNase activation. The cytoplasmic side of yeast IRE1 harbors a kinase domain and a globular domain designated a kinase-extension nuclease (KEN) domain. Similar to other typical kinases, it contains a bi-lobal fold spanning from an N-terminal lobe (N-lobe) to C-terminal lobe (C-lobe). The KEN domain exclusively contains α helices, which are interconnected by short loops, and an ATP binding pocket lies between the N-lobe and C-lobe (Fig. 2) [40]. In this crystal structure, the kinase domain of the IRE1 homo-dimer followed a back-to-back arrangement with two-fold symmetry, in which IRE1dimer is oriented facing opposite to each other in a manner that kinase active sites face outwards, impeding transphosphorylation, and giving rise to an oligomeric RNase active complex [40,41]. The crystal structure of human IRE1 revealed similar structural features, however there was a presence of an extra helical domain merged with the C-lobe. Also, it showed a face-to-face arrangement, when trapped with ADP at the active site [42]. This model proposes that the release of Bip positions the cytoplasmic kinase domain in a face-to-face orientation. In this orientation, the flexible activation segment of one monomer might possibly traverse to the catalytic centre of an adjacent monomer [42]. An interesting observation came from the structural studies carried out with apo human IRE1. It revealed that, similar to yeast IRE1, human IRE1 orients in a back-to-back arrangement, however with some structural differences [43].

Models, presented for the mechanism of sensing unfolded proteins show a divergence from yeast to human. However, due to the conservation of mechanism of action, it plausible that their ER stress sensing methods follows a similar program. This discrepancy is partially resolved by a novel model, unifying activation model put forth by Peter Walters group [39]. This model proposes that different structures obtained for yeast and human IRE1 represent different stages of the IRE1 oligomerization dynamics. By performing biochemical and structural approaches, their study revealed that like yeast IRE1, human IRE1 directly interacts with peptides through its MHCI like groove. Bindings of peptides to a hydrophobic patch at groove induce conformational changes and promote oligomerization. These results together with others put forth a model where a face-to-face dimer organization represents an intermediate conformation, while the active state of IRE1 is denoted by back-to-back dimer arrangement [4,39,44].

IRE1 oligomerization directly regulates its transautophosphorylation and this activation coupled with RNase activation induces conformational changes and structural rearrangement of the RNAse domain, consequently leading to its activation [43]. The structural studies of the IRE1 oligomer revealed the formation of two extra interfaces: Interface 2 (IF2), where the RNase domains of monomers make contact in twofold symmetry (IF2) and Interface3 (IF3) created by a filamentous arrangement of monomeric kinase domains. The IRE1 oligomer displays similarities to a double helical DNA structure, where IF1 and IF3 represent base pairing of nucleotides and phosphodiester linkages respectively [41]. Furthermore, the 3-D visualization of the IRE1 oligomer pointed out additional structural elements like closed loops, branch points and ring-like features [6]. The RNAse domain contains a structural element, which is important for its catalytic activity, designated as the helix-loop element (HLE). The stability of HLE motifs varies within different structures of the IRE1 dimer; in some it is distorted, while in others it is unresolved. However, in an oligomeric state the HLE motif is in a highly stable and resolved form and accessible to the substrate [39,40,45,46].

2.3. Lipid sensing and IRE1 activation

The properties of the lipid bilayer are also important for sensing unfolded proteins and activation of IRE1. Earlier studies demonstrated that IRE1 was involved in the biosynthesis of inositol [47]. The requirement for IRE1 in the absence of inositol (a precursor for phospholipid synthesis) was not clear until a study reported that ER stress is directly connected to defective lipid biosynthesis [32,48,49]. A double knockout of lipid biogenesis genes Orm1 and Orm2 result in UPR induction [50]. Alternatively, IRE1 can be activated in response to inositol depletion, which is independent of sensing unfolded proteins in the ER [51,52]. Deletion of genes associated with the lipid component of the membrane creates membrane aberrancy, which is detected by the cytosolic domain of IRE1 leading to its activation [52]. Similarly, distorted ER membrane morphology activates IRE1 [53]. Several recent studies have demonstrated the role of fatty acid saturation in UPR induction. For example, treatment with the increased levels palmitate activates IRE1 [54]. Palmitate does this effect by increasing the saturation of the ER membrane, which is actually known to contain low saturated phospholipids [55,56]. The effect was enhanced by inhibiting Stearoyl-CoA desaturase-1 (SCD1) [54]. SCD1 is responsible for converting saturated fatty acids to unsaturated fatty acids. Furthermore, inhibition of Sirtuin-1 (Sirt1), an upstream regulator of SCD1 also leads to the activation of UPR [57,58]. Lipid bilayer stress activates IRE1 independent of its lumenal domain [52,59]. This function of IRE1 is attributed to its amphipathic helix (AH) between 526 and 543, which is present within the trans-membrane helix (TMH) region [60]. A mini-substrate construct containing the AH and TMH regions (526–561) can respond to lipid perturbation and assemble in an oligomer like that of wild type IRE1, indicating the potential role of AH in ER stress sensing by IRE1 [60].

3. IRE1 mediated splicing

The process of RNA splicing is catalyzed by the spliceosome, a huge RNA-protein complex [61]. However, IRE1 catalyzes this reaction in a spliceosome-independent manner. IRE1 substrates, Hac1 in yeast and Xbp1 in mammals, were independently identified [8,62–64]. Both Hac1 and Xbp1 follow somewhat similar mechanisms of splicing, however due to variances in intron architecture, different methods and factors are involved in the catalytic pathway. For example, no unspliced protein product from unspliced transcript is formed in yeast, in contrast to humans. The Hac1 intron is 252 nt in size and makes a loop-like structure that blocks translation, while the Xbp1 intron is just 26 nt long and therefore is unable to form any loop kind of structure to block translation [2,65]. Therefore, detailed knowledge of the IRE1 splicing machinery operating in yeast and mammals would be of great importance to gain a thorough understanding about diversity in IRE1-mediated splicing.

3.1. IRE1 splicing targets

IRE1 is a conserved transmembrane protein, which transmits its signal through activation of a transcription factor. The immediate target of IRE1 in yeast is Hac1, which was independently identified by two research groups [62,63]. It was observed that Hac1 binds to the unfolded protein response element (UPRE) in the nucleus [66,67]. IRE1 activates Hac1 mRNA in an unconventional way. It catalyzes non-canonical splicing by removing a 252 nt intron; consequently, this results in the production of a mature protein that differs from the unspliced form at the C-terminal end [68]. Indeed, expression of intron-

less Hac1 from its own promoter can activate UPR independent of stress conditions [62]. Spliced Hac1 encodes a protein of 238 amino acids, whereas unspliced Hac1 encodes a protein of 230 amino acids. Only the ER stress-driven spliced product of Hac1 was detectable in cell lysates. Therefore, it was proposed that splicing of Hac1 leads to the formation of a stable product while the unspliced product is highly unstable and is degraded by the ubiquitin-proteosome pathway [62,63]. In contrast, it was proposed that lack of detection of unspliced Hac1 is not due to instability of its protein, but rather intronic features of Hac1 mRNA that are able to block its translation [2,69]. This view was supported by another study, where they showed that interaction between the intron and its 5'untranslated region blocks mRNA translation [70]. In the mammalian system, Xbp1 was identified as the IRE1 splicing target, and it represents the Hac1 homolog [8,64]. It was shown that splicing of Xbp1 is similar to that of Hac1, but, in contrast to Hac1 mRNA, Xbp1 is being translated at a low level as the Xbp1 intron is just 26 nucleotides in length and would not be able to form any kind of loop structure to block translation [65].

3.2. Mechanism of IRE1 mediated splicing in yeast

IRE1-mediated splicing is carried out in a non-canonical way, unlike that driven by the RNA spliceosome machinery [67]. The splicing signals in Hac1 mRNA do not resemble the canonical sequences at the 5′ and 3′ ends of introns. Also, mutations in two main components of the spliceosome, Prp8 and Prp2 do not affect Hac1 mRNA splicing [71]. IRE1 cleaves Hac1 at the 5′ and 3′ ends of the intron, after which the exons are then joined by a tRNA ligase, concluding the splicing process [10] (Fig. 4a). Hac1 mRNA is predicted to contain four stem loops with the 5′ splice site located in stem loop1 and the 3′ splice site in stem loop 4. A branch-point-like sequence (UACUAAG) was also found in stem loop3, however, mutational analysis proved that this sequence is dispensable for splicing [72]. The 5′ and 3′ splice sites form similar stem-loop structures, which contain 7 nucleotides with a G residue at the third position [72]. A series of point mutants were generated by PCR-based mutagenesis to check the sequence specificity around the 5′ and 3′ splice junctions. Four nucleotides were found to be essential for Hac1 mRNA splicing, and these are conserved between the 5′ and 3′ splice sites [10,72]. Using mini-substrate constructs, it was revealed that the stem-loop structures of Hac1 mRNA are enough to recruit IRE1 and result in efficient RNA cleavage [9]. Therefore, it can be concluded that the sequence stringency and particular stem loop formation at the 5′ and 3′ sites is essential for efficient splicing of Hac1 mRNA.

Unlike canonical splicing, Hac1 mRNA splicing does not follow any sequential order for cleavage at the 5′ and 3′ splice sites, rather cleavage occurs randomly [9]. Generation of point mutations at the G residue of the 5′ site or 3′ site completely abolished Hac1 mRNA splicing. It was found that a 5′ splice site mutation blocked cleavage at the 5′ site only, as shown by the accumulation of the uncleaved 5′ exon-intron. Similarly, mutation at the 3′ splice site blocked cleavage at the 3′ splice site only [10]. In another study, it was found that mutation at the −1 position of the 5′ splice site could block cleavage at the 5′ splice site and

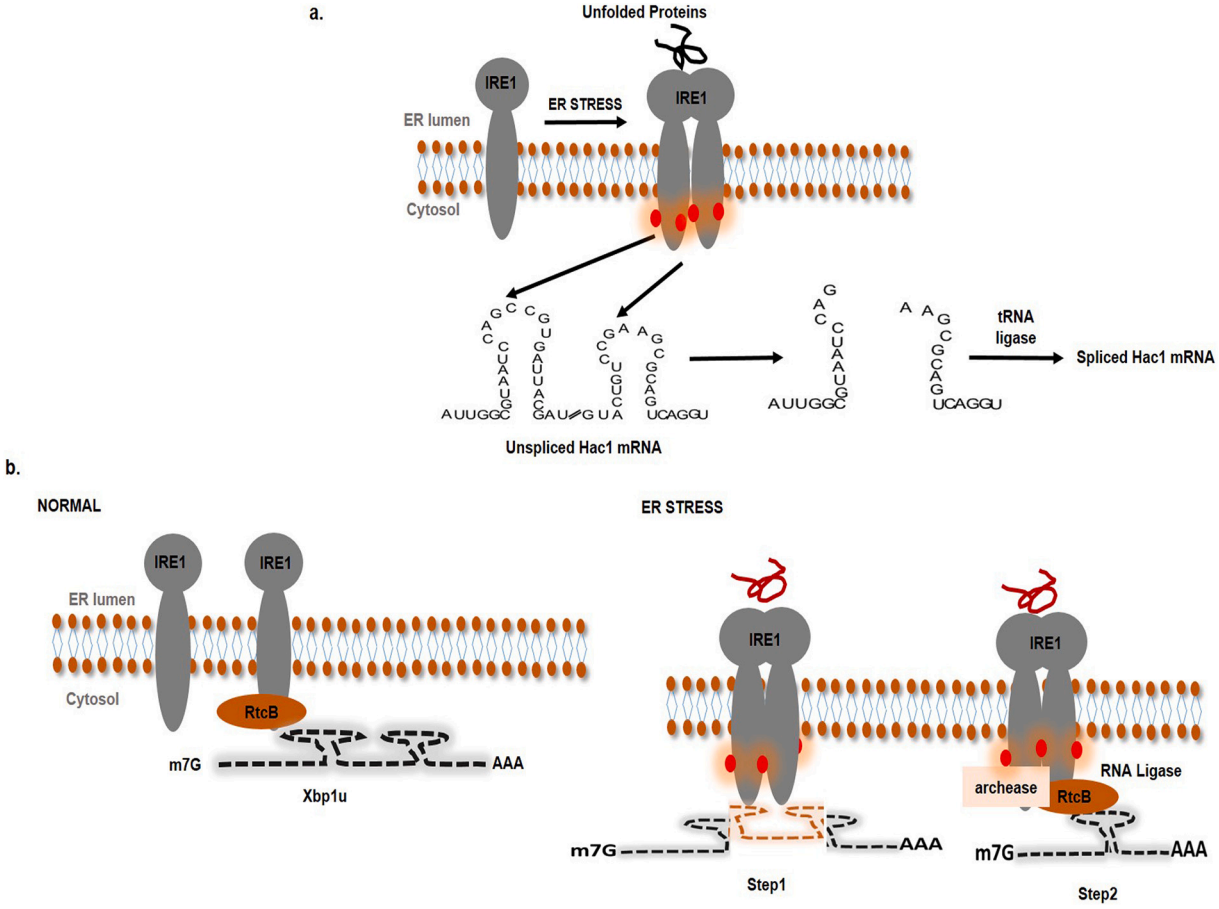

Fig. 4. Mechanism of IRE1-mediated mRNA splicing. a) The Hac1mRNA follows a non-conventional pathway, where activated IRE1 cleaves the left and right stem-loops between GC and GA, removing the intron. These cleaved products are then acted upon by a tRNA ligase that leads to the formation of spliced Hac1 mRNA. b) Under normal circumstances, Xbp1 unspliced mRNA and the tRNA ligase (RtcB) form a triad complex with the cytosolic domain of IRE1. Once unfolded proteins accumulate in the ER lumen, IRE1 through its endoribonuclease activity cleaves the Xbp1 mRNA. This is followed by the ligation of two cleaved products by RtcB resulting in the formation of functional Xbp1 mRNA.

mutation at −1 of the 3′ splice site blocks cleavage there [72]. These studies confirm the random order in Hac1 mRNA splicing and also demonstrate that the G residue represents the site for RNA cleavage at both the 5′ and 3′splicing site.

Labelling of 5′ and 3′ splice junctions revealed that IRE1 recruits and recognizes stem loop structures of Hac1 mRNA, and cleaves it at the 5′ splice site and the 3′ splice site while releasing 2′-3′ cyclic phosphate at the 5′ end and a free -OH group at the 3′ end [9]. It was found that all tRNA ligase homologues from *K. lactis*, *S. pombe*, and *A. thaliana* could ligate spliced Hac1 mRNA efficiently. It was also observed that a plant homolog of yeast tRNA ligase could not complement UPR activation due to failure in releasing translational attenuation. This study also pointed out that tRNA ligase remains bound to Hac1 mRNA through interaction with the translational machinery [73]. tRNA ligase phosphorylates the 3′ end of the 5′ exon as well as of the intron, as both contain free -OH groups, and it adenylates the 5′ end of the 3′ exon and then ligates the two exons [9]. The information stored within the intron of Hac1 mRNA is not sufficient to accomplish the splicing process. Other structural elements are required for the process [9]. For example, there is the presence of an extended stem-loop structure, which contains a bipartite element (BE). It was found that deletion of the 3′ BE resulted in reduction of mRNA splicing, while insertion of a 64 bp 3′ UTR region containing the 3′BE restored splicing of a reporter mRNA. The actual function of the 3′BE is targeting of Hac1 mRNA to the ER where IRE1 is localized. Similarly, the 5′ UTR is required for efficient targeting and translational attenuation of unspliced Hac1 mRNA [73,74].

3.3. Mechanism of IRE1 splicing in mammals

Initial studies by Yoshida et al. identified that in the mammalian systems, the IRE1 splicing substrate is Xbp1 [64]. To find out whether the process of Xbp1 splicing is similar to that of Hac1 mRNA, they did mutational analysis of sequences around the 5′ splice site and 3′ splice site. Mutations were created at the −3, −2, −1, +1 and +3 positions of 5′ and 3′ splice sites. Changes at the −3, −1, +3 positions affected splicing of Xbp1 mRNA, while changes at −2 of either the 5′ or 3′ splice site did not affect the process of splicing. In addition, variation at +1 aborted splicing completely, as this site (G) represents the site of cleavage as in Hac1 mRNA [64]. In agreement with this one, another study identified that Xbp1 mRNA splicing is very similar to that of Hac1 mRNA in yeast [75]. IRE1 lacking its luminal domain could splice Xbp1 mRNA, showing that IRE1 can catalyze splicing without activation by the UPR [75]. In addition, their study concluded that Xbp1 splicing might occur in the cytoplasm, but requires ongoing transcription for the initial phase [75]. A study involving homolog searches and mRNA alignment revealed that the intronic features of Xbp1 are similar to that of Hac1 mRNA [76]. These results together suggest that Xbp1 mRNA splicing is highly similar to that of Hac1 mRNA.

Like in Hac1, Xbp1 splicing generates 2′, 3′ cyclic phosphates and 5′ -OH end [77], hence creating a need for mammalian tRNA ligase to complete the splicing of Xbp1 mRNA. To identify this mammalian counterpart of yeast tRNA ligase, Lu et al., utilized a synthetic biology approach. In a synthetic genetic screen against Xbp1, they identified mouse RtcB, an ER-localized enzyme as the main mammalian tRNA ligase, which is involved in completion of Xbp1 mRNA splicing [78]. It was reported that RtcB could form a complex with IRE1 and was possibly localized to the ER. Due to the close association of IRE1 and RtcB, the two steps of Xbp1 mRNA splicing are coupled and preferentially occur in the ER [78]. In another study, it was found that RtcB efficiently binds to Xbp1u mRNA [79]. Based on these results a model was proposed for Xbp1 splicing, according to which before IRE1 activation RtcB might be attached in a triad complex with IRE1 and Xbp1u mRNA. Upon activation, IRE1 cleaves Xbp1 mRNA and RtcB joins the two ends (Fig. 4b) [78,79]. A co-factor of the tRNA ligase complex, the archease was identified to be important for Xbp1 mRNA splicing. This study concluded that complete abrogation of Xbp1 mRNA splicing requires the absence of both RtcB and archease [80]. A study was carried out with a yeast mutant strain rlg1–100 to functionally characterize mammalian tRNA ligase [81]. It was found that only upon co-expressing both RtcB and archease, mammalian tRNA ligase could efficiently complement splicing in yeast cells. This mammalian tRNA ligase system could efficiently ligate Hac1 mRNA without Xbp1, though Hac1 mRNA do not contain any potential binding site for RtcB. These findings suggest that interactions between tRNA ligases and their corresponding substrates might not be sequence specific but rather IRE1 mediated [81].

Xbp1 mRNA like its counterpart in yeast should be localized to the ER to be efficiently spliced as IRE1 is primarily located in the ER. Initially, it was observed that several mRNAs including Xbp1 are localized to the ER membrane [82]. The membrane localizing property lies in the protein version of unspliced Xbp1 mRNA. Xbp1u (Xbp1-unspliced) protein contains a hydrophobic region HR2 (hydrophobic region 2) that is important for its association with the membrane [83]. Once this protein is localized to the ER, it recruits the unspliced Xbp1 mRNA to the membrane. Deletion of the HR2 region not only abolished the distribution of Xbp1u protein to the membrane but also inhibited recruitment of Xbp1u mRNA [83,84]. In this study, a model was proposed, which posits that the Xbp1 mRNA is recruited to the membrane as a part of R-RNC (mRNA-ribosome nascent complex) through translation of Xbp1u [83]. HR2 has been shown to contain features similar to a type II transmembrane domain that results in insertion of Xbp1u into the ER membrane [85,86]. Besides, it was found that translational pausing is important for recruitment of Xbp1u R-RNC complex to the membrane [87]. The C-terminal part of Xbp1u protein harbors a conserved peptide region of 26 amino acids, which is important for translational pausing. The authors observed that translational pausing mutants reduced splicing of Xbp1u mRNA. Thus, it was concluded that translational pausing is important for recruitment and efficient splicing of Xbp1u mRNA [87]. Trypsin digestion halted Xbp1u R-RNC recruitment to the membrane, which implies that some protein factors of the ER might be involved [88]. The factor involved was found to be SRP (the signal recognition protein), which captures HR2 of the Xbp1u protein and then translocates Xbp1u R-RNC to the Sec61 translocan, which is bound to the IRE1 protein. This co-translational translocation of Xbp1u mRNA to IRE1-sec61 might enhance the efficiency of splicing [88]. In a similar study, it was observed that the Xbp1u R-RNC complex is passed on to the Sec61 translocon through an interaction with SRP. Translational pausing exposes HR2 of Xbp1u protein and allows it to bind with SRP [89]. Strikingly, the important criterion for efficient association of SRP with HR2 of Xbp1u R-RNC is the particular distance of HR2 from a translational pausing site and the specific ribosome configuration created by pausing. Also, it was suggested that splicing of Xbp1u mRNA occurs on the translocon [89].

4. Regulated IRE1 Dependent Decay

Xbp1 independent RNase activity of IRE1 known as RIDD is a phenomenon in which a subset of ER-localized mRNA is degraded by IRE1 upon ER stress [12]. It was originally discovered in Drosophila S2 cells through gene profiling experiments and later reported in mammals and fission yeast; although the levels of mRNA degradation were much less in these organisms as compared to S2 cells [12,90,91]. In the mammalian system, IRE1 overexpression or IRE1 hyper-activation through chemical inducers resulted in the activation of RIDD [11,92]. Both isoforms of IRE1; α and β, identified in mammals are capable of RIDD as well as Xbp1 splicing, but IRE1β is stronger at exerting RIDD activity as compared to its paralog IRE1α [91]. Binding of 1NM-PP1 (ATP analog) to a mutant version of IRE1 can induce Xbp1 splicing activity independent of ER stress. However, RIDD is functional only after the induction of ER stress, pointing towards a plausible difference between Xbp1 splicing and RIDD [92].

4.1. Substrate selectivity of RIDD

RIDD targeting has been solely dedicated to ER-localization of the mRNA products because co-translational translocation of these proteins will bring their mRNAs close to IRE1, increasing the chances of degradation [12,93]. The majority of mRNAs targeted by RIDD belong to the family of signal peptides and transmembrane domains that would represent an additional burden to the folding machinery of ER during stress [14]. The deletion of the signal peptide from known RIDD targets prevents its degradation, while the addition of the same signal peptide even to GFP promotes its degradation by RIDD [93]. However, there are a few exceptions, like PlexinA mRNA that is strongly associated with the ER membrane and is still protected by RIDD degradation even during ER stress. The mRNA gets continuously translated and protected from degradation due to the presence of an upstream regulatory ORF [93]. Likewise, Smt3 mRNA is a homolog of SUMO (Small ubiquitin-like modifier), which does not associate with the ER membrane, but is still a target for RIDD and is cleaved on the stem-loop structure present on its mRNA [94]. Because of the variability between mRNAs expressed in different systems and the massive remodeling of transcription that occurs during the UPR, compiling a comprehensive list of RIDD targets has remained challenging. However, of the confirmed RIDD targets in mammalian cells, almost all have a similar consensus sequence CUGCAG and a predicted stem-loop structure that is similar to stem-loop of Xbp1 mRNA (Fig. 5a) [92–96]. Furthermore, RIDD can be turned off by introducing a mutation in the consensus sequence or by deleting the stem-loop structure [96].

About 37 IRE1 substrates have been published to date, and the cleavage sites for all of them have been mapped. All the identified cleavage sites resemble that of Xbp1 making RIDD highly sequence-specific [96]. Among the 37 putative RIDD substrates, some of the most common ones include BLOS-1, SCARA3, COL6a1, Hgsnat, Sparc, Sumo, and 28s rRNA [11,97]. The majority of RIDD substrates, which account for around 64% are ER-localized whereas the other 36% include cytosolic and nuclear fractions [97,98]. The efficacy of RIDD targeting demonstrates a cell type-specific correlation and also with the type of ER stress inducer [99]. For example, Blos1 gets more efficiently degraded in DTT treated cells compared to Tg treated cells. Moreover, Blos1 showed different levels of degradation between Hek293 and HepG2 cell lines [99].

4.2. Structural features of mRNA essential for RIDD targeting

The cleavage sites for most of the RIDD substrates coincide with the cleavage site of Xbp1 mRNA (Fig. 5a) [99–101]. For example, degradation of Blos1 depends on a stem-loop structure (a seven-nucleotide (nt) loop with the four conserved residues) that is followed by a shorter stem of four consecutive bases (allowing for AU, GC, and GU to pair) [8,99]. Mutagenesis experiments revealed that a single base-pair mutation at the IRE1 cleavage site from G to C and at the second conserved pair residue abrogates RIDD is the case of Blos-1 [99]. Similar trends were observed in the case of BLOC1S1 and angiopoietin-like protein 3 (Angptl3) upon mutation of G to C at the site of cleavage [100,101]. Additionally, ligation of a stem-loop structure at the 3′ UTR of GFP resulted in the degradation of the reporter in IRE1 dependent manner [99]. However, there are discrepancies to this rule in the case of Hgsnat; wherein the mutagenesis at second stem-loop does not affect its degradation [99]. This stability in Hgsnat mRNA can be due to a shorter stem-loop and fewer GC pairs than the first stem-loop. This data suggest that the sequence and stability of the Xbp1 like step loop are prerequisites for RIDD [99–101].

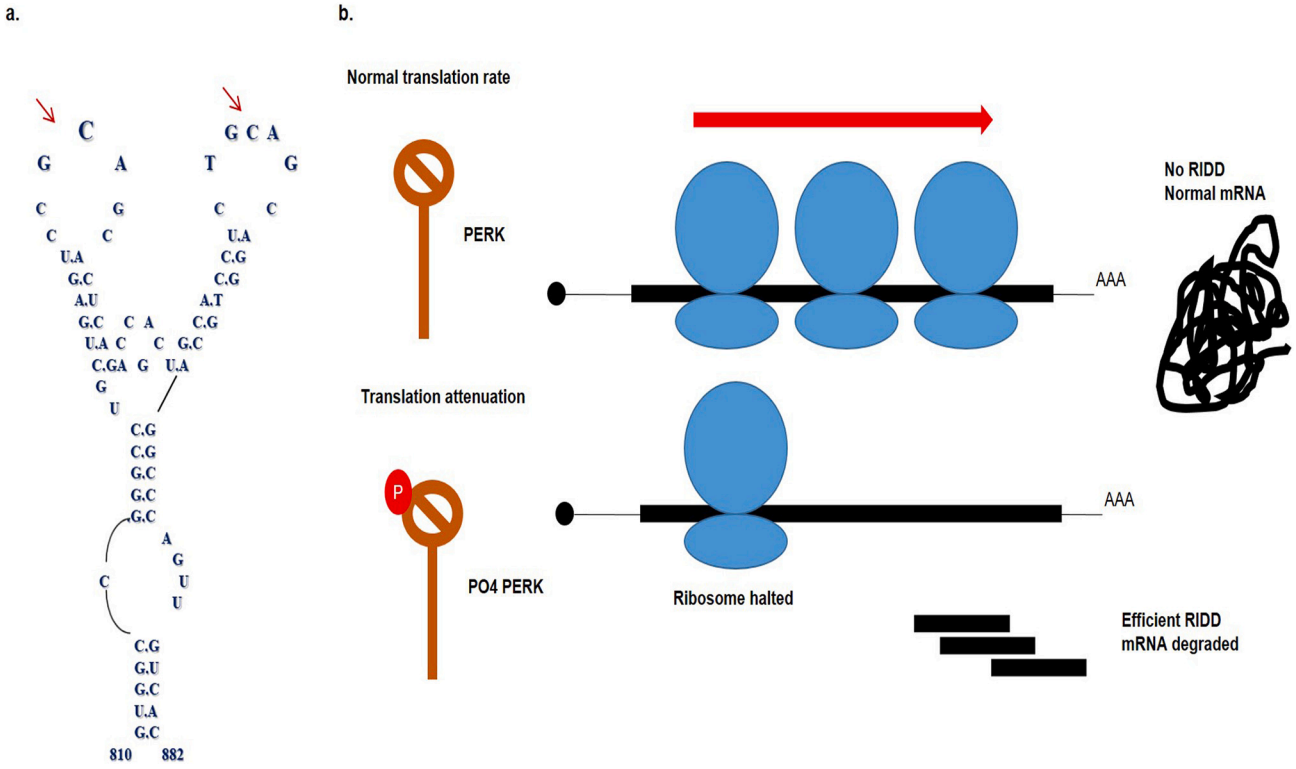

Fig. 5. RIDD targeting and its regulation. a) An Xbp1 like stem-loop (SL) is both necessary and sufficient for RIDD in mammalian cells. RNA from Hgsnat, Blos1, and other RIDD targets contain these SLs. Red-colored arrows indicate putative IRE1 cleavage sites. b) During normal translation, PERK is unphosphorylated and ribosomes bind the mRNA leading to normal mRNA synthesis hence RIDD is not seen. However, during ER stress conditions, PERK gets phosphorylated leading to a state where ribosomes get halted on the mRNA. This ensures that IRE1 gets enough time to degrade the mRNA via RIDD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. PERK mediated regulation of RIDD

PERK pathway has been shown to regulate the RNAse activity of IRE1 [94,99,102]. Several studies suggest that PERK acts as a positive regulator of RIDD [94,99]. These studies showed that the depletion of PERK by siRNA knockdown results in the inhibition of RIDD in Blos1 and Col6a1 mRNAs. This effect was reversed by artificial attenuation of translation [94,99]. It points out that the attenuation of translation initiation mediated by PERK, creates a condition wherein the RIDD mRNA targets are more accessible to degradation by IRE1. In contrast, under PERK depleted conditions, translation is restored, and the ribosomes physically occupy the mRNA, which indirectly limits the access of IRE1 to degrade the target mRNA (Fig. 5b) [94,103,104]. There are some RIDD targets, which show insensitivity to PERK depletion, like Hgsnat [99]. Hgsnat has decreased translational efficiency, which is attributed to the presence of rare codon at the 5′ end of its transcript that allows for RIDD to proceed normally even during translational attenuation. These rare codon clusters are absent in the 5′ regions of PERK-sensitive RIDD targets [99]. Furthermore, treatment with Cycloheximide resulted in decreased levels of Blos1 and Col6a1 mRNA [99]. Together, these studies highlight the role of PERK mediated translational attenuation in RIDD either by decreasing the occupancy of ribosomes on target mRNA or by stalling ribosomes on target mRNA that blocks eukaryotic translational elongation [94,99]. In contrast, a recent study advocates for the opposite role of PERK in RIDD activity [102]. The study revealed that PERK is responsible for the de-phosphorylation of IRE1 during terminal stages of ER stress. This activity of PERK is independent of ATF4 but operates through RPAP2 phosphatase [102]. RPAP2 physically interacts with IRE1 and brings about the dephosphorylation of its activation loop corresponding to residue Ser724, 726, and 729. This was followed by inhibition of RIDD activity as found in the case of Blos1, CD59, DR5, etc. [102]. To resolve the inconsistencies between differential activities of PERK towards RIDD, it is intriguing to consider the possibility that the PERK activity is dependent on the strength and time course of ER stress [105]. Initially, PERK acts as a positive regulator of RIDD by attenuating translation through eIF2α phosphorylation [99,106]. But at later stages, eIF2α signaling is aborted through GADD34 [107,108], and PERK now turns to a negative regulator of IRE1 signaling. It attenuates IRE1 phosphorylation through RPAP2 phosphatase and consequently inhibits RIDD activity [102].

5. Functional duality of IRE1

IRE1 has been established as a key player in regulating cellular homeostasis in response to ER stress [4]. This signaling pathway needs to be terminated for apoptosis to take place [102,109]. But, under chronic ER stress conditions, IRE1 is responsible for initiating the cell death pathway [92,98]. This functional duality of IRE1 arises due to its RNase activity. Differential RNase outputs of IRE1 work either in a synergistic or antagonistic manner depending on the extent and duration of ER stress, to regulate cell fate [13]. The IRE1 dual functionality is also determined by the state of the cells and is governed by various protein factors that communicate with different cellular pathways [110].

5.1. Models for distinctive IRE1 RNase activity

IRE1 ribonuclease activity has two outputs, which operate differentially [13]. Several models have been postulated to explain the differential RNase activity of IRE1, but none of them is yet inclusive. An initial attempt was made by the Feroz Papa group to elucidate how RIDD could be decoupled from XBP1 splicing [92]. They used a kinase-dead mutant of IRE1 (I642G) and found that it can activate RNase in a way that it will only catalyze Xbp1 splicing but not RIDD. It has been established that 1NM-PP1 [1-tert-butyl-3-(naphthalen-1-ylmethyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine] bypass the auto-phosphorylation for Xbp1 splicing in I642G mutant [111,112]. It was proposed that a dimeric moderately active RNase pocket is sufficient to catalyze Xbp1 splicing as Xbp1 represents the preferred substrate for IRE1. For RIDD activity there is a need for higher-order structure with an oligomerized kinase/RNase domain that could be achieved only through phosphotransfer activity of IRE1 [92,113]. They speculated that the I642G mutant fails to attain a higher-order structure. Besides, lower concentrations of IRE1α type II kinase inhibitors, which prevent IRE1 oligomerization allow Xbp1 splicing but abrogates RIDD activity [113,114]. According to this model, Xbp1 splicing requires the specific activity of the RNase domain of IRE1 while for RIDD it represents promiscuous RNase activity [92,113].

Another study presented a contrary view to the previous model [115]. As per this model, it was found that for Xbp1 splicing higher-order structure of IRE1 is required while in case of RIDD monomer or dimer is sufficient. In addition, when they analyzed L745G yIre1 a yeast homolog of mammalian I642G IRE1 mutant for RIDD activity in vitro, they surprisingly found that L745G yIRE1 upon binding to 1NM-PP1 performs RIDD activity [115]. This finding is substantiated by another study, where it was found that in the presence of 1NM-PP1, I642G IRE1 showed RIDD activity [98]. It was observed that expressing I642G IRE1 in ire1−/− cells restores RIDD activity [11]. These results conclude that the IRE1 splicing activity is conserved and preferred over RIDD.

Additionally, phosphorylation at different residues might uncouple RIDD from Xbp1 splicing. Xbp1-deficient cells show increased levels of IRE1 and pronounced RIDD activity [116,117]. These cells also show increased phosphorylation of S729 compared to S724 and S726 [118]. In response to BFA and Tu, Xbp1 splicing can be observed even in the absence of S729 phosphorylation. It was further demonstrated by KIRA6, which specifically inhibited S729 phosphorylation in response to subtilase cytotoxin (SubAB), but it did not inhibit Xbp1 splicing. However, phosphorylation at S729 residue is imperative for RIDD activity. These results reveal that the phosphorylation status of IRE1 might be involved in differentiating substrate specificity between RIDD and Xbp1 [118].

Apart from the models presented above, there is an alternative model to explain the distinctive RNase activity of IRE1. In this model, there occurs an assembly of a huge protein complex on IRE1 called UPRosome [110,119]. This protein platform regulates multiple IRE1 signaling networks in a selective and specific manner. UPRosome might provide a dynamic space where distinctive RNase activity of IRE1 could be regulated [120]. For example, factors related to RNA degradation machinery might be in association with IRE1 directing it towards RIDD [121,122], while the interaction of IRE1 with tRNA ligase (Trl1/RtcB) leads to preferential activation of Xbp1 splicing [123,124]. So, identifying such elements, which are involved in the regulation of its differential RNase activity, would have great importance for cell biologists.

5.2. RIDD in the differential IRE1 functioning

RIDD plays a dual function, maintaining homeostasis during low ER stress as well as induction of cell death via apoptosis during irreparable ER stress [125,126]. Maintenance of ER homeostasis is a task ascertained to basal RIDD activity, which is defined as detectable RIDD activity in the absence of Xbp1 splicing [97]. Multiple mechanisms are indicative of the fact that RIDD is required for ER homeostasis. For example, in case of metazoans, the load on ER is alleviated by general mRNA degradation [11,12,92] and by cleavage of 28s rRNA [127], which leads to inhibition of global protein synthesis. Studies from *S. pombe* have also determined that RIDD assists in the reduction of protein influx into the ER by around 15% [91] thereby, relieving the protein load on ER. Further evidence of the role of RIDD in maintaining cellular homeostasis is provided by mutational studies. It has been shown in *C. elegans* that ER homeostasis is altered in both the mutants of Xbp1 and IRE1, but the extent is greater in IRE1 mutants suggesting a role of RIDD in ER homeostasis [122,128]. The mRNA decay machinery such as the exosome complex and proteins involved in nonsense-

mediated mRNA decay (NMD) is involved in maintaining ER homeostasis since both of them are used by RIDD for rapid decay of mRNA. This suggests some indirect roles of RIDD in maintaining homeostasis [122]. Besides, numerous studies are indicating a cytoprotective role of RIDD in metazoans; for example, it protects liver cells from acetaminophen-induced toxicity by degrading P450 cytochrome variants [95] and also protects pancreatic beta cells by modulating insulin levels during hyperglycemia [129]. RIDD displays cytoprotective activity in dendritic cells by reducing the levels of translation substrates [130]. RIDD also promotes cell survival of Xbp-1 deficient cells in vesicular stomatitis virus (VSV) and herpes simplex virus (HSV) infections [131]. RIDD degrades miR-125a, a pro-apoptotic regulator, which down-regulates the expression of BCL2 and BCL2L12 [131,132]. This effect was reversed by reconstitution of miR-125a [131]. All these studies cumulatively emphasize on the homeostatic role of RIDD in cells.

Under chronic ER stress, RIDD operates to bring apoptosis of cells mainly by degrading the set of mRNAs, which are otherwise essential for survival. RIDD causes degradation of micro-RNA miR-17, which governs the post-transcriptional regulation of TXNIP (Thioredoxin interacting protein) during ER stress [133]. TXNIP is a mediator of cell death and comes into play during irremediable ER stress levels, a process termed as terminal UPR. Upon the degradation of miR-17, the mRNA of TXNIP gets stabilized, which leads to up-regulation TXNIP protein [133]. Increased TXNIP levels activate the NLRP3 inflammasome [134]. The NLRP3 inflammasome is a multimeric protein complex, which initiates the death-signaling cascade by recruiting procaspase1 via an adaptor protein known as apoptosis-associated Speck-Like Protein Containing CARD (ASC) [133,135]. Additionally, the depletion of NLRP3 leads to suppression of cell death in HUVECs in response to ER stress [135]. RIDD also promotes apoptosis in a Caspase-2 dependent manner [98]. During normal conditions, microRNAs miRs 17,34a, 96, and 123b destabilize Caspase2 mRNA and prevent its translation. However, during high ER stress, the rapid decay of these selective miRNAs via the RIDD pathway causes de-repression of Caspase-2 mRNA and a sharp increase in the protein levels leading to the activation of executioner caspases and hence apoptosis [98]. Thus, IRE1 regulates the translation of pro-apoptotic proteins through RIDD dependent microRNA decay.

5.3. Xbp1 as a fate executer

Active Xbp1 translocates to the nucleus and activates a plethora of genes involved in cellular homeostasis [8,10]. These genes code for proteins involved in ER-associated degradation (ERAD), chaperons, lipid synthesis, protein folding, and maturation. Xbp1 is an established homeostatic regulator of the IRE1 signaling pathway [174,175]. For example, Xbp1 −/− livers display increased apoptosis, and Xbp1 −/− mouse embryos show embryonic lethality [136,137]. This lethality can be rescued by the introduction of Xbp1 transgene [136]. Similarly, Xbp1 −/− Rag2 −/− chimeric mice suffer from loss of dendritic cells [138]. However, Xbp1 has been shown to execute an unusual trait under chronic ER stress conditions [139]. Xbp1 generally binds to UPRE sequence TGACGTGG and activates its usual downstream targets [140], but upon accumulation, it changes its promoter binding specificity and binds to a non-canonical promoter element TGACGTGA present in KLF9 [139]. Subsequently, KLF9 then activates transmembrane protein 38B (TMEM38B), and inositol 1,4,5-trisphosphate receptor type1 (ITPR1), membrane ion channels genes. These proteins buildup the cytoplasmic calcium levels in the cells because the depletion of either of the protein resulted in decreased cytoplasmic calcium levels [139,176,177]. It is also known from earlier studies that calcium release from ER to cytoplasm promotes cell death [141]. Cells treated with tunicamycin in the absence of TMEM38B or ITPR1 show decreased cell death. Additionally, KLf9 knockout mice showed decrees in ER stress markers when challenged with tunicamycin compared to the wild type [139]. These results reveal that Xbp1 displays differential activity in response to the varied strengths of ER stress. Newcastle disease virus (NDV) induces apoptosis through the induction UPR. Interestingly Xbp1 splicing remains active, and its inhibition leads to a decrease in the production of apoptotic markers [142]. Consistent with this, Vesicular stomatitis virus (VSV) and Herpes simplex virus (HSV) infections require active Xbp1 to induce cell death, which introverts in Xbp1 knockout conditions [131] Xbp1s supplementation in endothelial cells (EC) lead to decrees in VE-cadherin levels, which induces apoptosis [143]. Xbp1 performs this action by modifying the acetylation and methylation status on the VE-cadherin promoter. Xbp1 induced apoptosis can be partially rescued by caspase inhibitors, which demonstrates that Xbp1 might induce activation of caspases in EC cells [143]. Macrophages show induction of IRE-Xbp1 in response to Nitrous Oxide (NO) followed by decreased viability [144]. Moreover, Xbp1 depletion in aged macrophages reduces apoptosis [145]. Reduced Xbp1 levels lead to a decrease in Bip production that leads to hyper-activation of IRE1. Due to unknown mechanisms, this hyperactive IRE1 is important to maintain aged macrophages [145].

5.4. Protein-protein interactions scrutinize IRE1 functions

Earlier, it was presumed that the IRE1 signaling pathway transduces in a unidirectional manner as IRE1-Xbp1-downstream effectors [8,32]. This notion was rebutted by various studies with the evolution of the idea that IRE1 acts as a platform to orchestrate with other signaling pathways [146–148]. Several proteins acting either as inhibitors or co-factors of IRE1 have been found to interact and modulate the IRE1 pathway, the concept that introduced 'UPRosome' as a signaling platform (Fig. 6) [110]. UPRosome can be used to define the broader contribution of IRE1 in various cellular pathways; it can also transmit information independent of IRE1's RNase activity. IRE1 kinase signaling is a linear pathway exclusively dependent on the RNAse activity of IRE1. The concept of UPRosome envisioned that IRE1 serves as a scaffold interacting with many proteins, which one or other way modulate it or to connect with other pathways to regulate its divergent signaling outputs [109]. Of note, the interaction of IRE1 with JNK and its associated protein mediate IRE1-dependent apoptosis. The cytosolic region of activated IRE1 interacts with the adaptor protein TNFR-associated factor 2 (TRAF2), triggering the activation of the apoptosis signal-regulating kinase 1 (ASK1) and cJun-N terminal kinase (JNK) pathway, thereby driving a cell towards apoptosis under irreparable damaged conditions [149,150]. Another feather added to the IRE1-TRAF2 module is AIP1 (ASK1-interacting protein 1), which interacts with both IRE1 and TRAF2. AIP1 promotes IRE1 dimerization by binding through its PH domain and facilitated apoptosis through ASK1-JNK activation [148]. JNK driven apoptotic activation is also influenced by a ubiquitin ligase, RNF13 (RING finger protein 13), and Ubiquitin D through its interaction with IRE1 [151,152]. IRE1, through its interaction with Nck adaptor protein, also communicates with Nuclear Factor-κ (NF-κ) signaling pathway, thus reinforcing the commencement of apoptosis during elevated stress [153]. In addition, the cytosolic ABL kinases, while localizing to the ER membrane, binds with IRE1 to rheostatically enhance its RNase activity, thereby potentiating ER stress-induced apoptosis [154]. The pro-apoptotic members of the Bcl-2 protein family have a role in promoting the IRE1 signaling pathway. Bcl-2 family members, BAX, BAK, BIM, and, PUMA, are known to increase the amplitude of the IRE1 signal [155,156]. These interactions might serve as a template to drive the homeostatic–apoptotic switch of IRE1 under chronic ER stress conditions [156].

The other set of proteins engage with IRE1 to promote its pro-survival function. Fortilin, a pro-survival molecule, interacts with the cytoplasmic domain of IRE1α, inhibiting its kinase and endoribonuclease (RNase) activity, thereby protecting cells from apoptosis [157]. Protein kinase C substrate 80K-H (PRKCSH) interacts with IRE1 to enables its oligomerization and activation. PRKCSH leads to the constitutive activation of Xbp1 splicing to promote the survival of cancer cells against ER stress toxicity [158]. Besides, a number of proteins have been found to physically engage with the IRE1, aiding to its structural

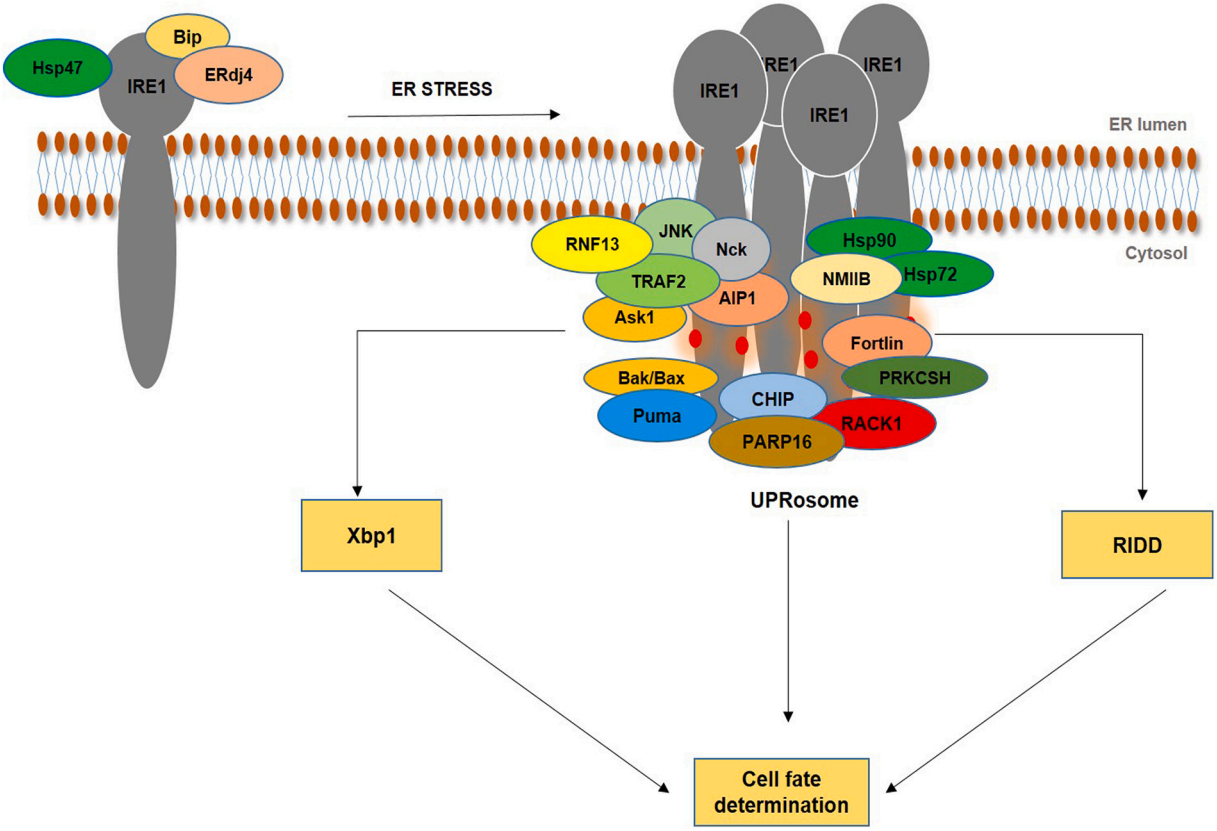

Fig. 6. UPRosome, a dynamic regulatory platform. An array of proteins physically interacts with the IRE1 either serving as its modulators or functional links with other pathways or simply the structural stabilizers; forming a structural platform known as UPRosome. UPRosome responds to ER stress either through RNase activity of IRE1 that is Xbp1 splicing and RIDD or through protein-protein interactions. The UPRosome acts as a dynamic centre, which regulates IRE1-dependent cell fate decisions.

stability. Cytosolic chaperones heat shock protein 72 (HSP72) [159], HSP90 [160], and cytoskeleton proteins actin and Non-muscle myosin-IIB (NMIIB) have a role in stabilizing the IRE1 structure [161]. Another molecular chaperone (HSP47) directly binds to the luminal domain of IRE1, facilitating its oligomerization in the adaptive phase of UPR. HSP47 acts as a stress sentinel, thereby setting a threshold for UPR activation [162]. The post-translational modifications also regulate the IRE1 activity according to the cell scenario. IRE1 phosphorylation is carried out by protein kinase A (PKA) [163] and dephosphorylation by PP2A with the aid of scaffold protein RACK1 [164]. PARP16 carries out the ADP-ribosylation of IRE1 [165], while CHIP has a role in the induction of IRE1α ubiquitination [166]. These protein-protein interactions serve as a convergent point of different pathways, which cumulatively sends pro-death or pro-survival signals. Thus, multiple checkpoints fine-tune the activity of IRE1 and, therefore, its functions in accordance with the cellular conditions.

6. Future of IRE1 signaling

Our understanding of the IRE1 signaling has considerably enhanced with the identification of several protein factors that interact with IRE1 to determine the extent and dynamics of its response [167]. The exact stimulus, time, and position that drive the organization of these specific IRE1 regulatory groups on the ER membrane are unclear. The strongest evidence for specific localization of IRE1 on the ER membrane comes from the MAMs (mitochondrial associated-membranes), the structural features that facilitate IRE1 signaling across the cellular organelles [168,169]. Furthermore, a recent study attempted to understand the IRE1 clustering where assembly and disassembly of IRE1 oligomers on the ER membrane is critically determined by the amplitude and timing of stress [6]. The study also suggested that IRE1 might be forming specific spatial arrangements on ER that are enriched with IRE1 protein and exclude other ER specific components [6]. This type of quantitative studies can be further extrapolated to investigate whether different protein clads interact with IRE1 at a specific time and location on the ER membrane to form varied regulatory microdomains. It will be helpful in understanding the intricate behavior of IRE1 under diverse cellular environments.

Yet another anonymous feature of IRE1 signaling is the heterogeneous response of individual cells towards ER stress. It has been reported that an asynchronous population of cells when subjected to ER stress does not respond similarly, indicating that cellular response is strongly guided by the state of the cell, its microenvironment, and cell cycle progression [6]. Further, studies showed that the downstream effectors of IRE1 could behave differently depending upon the cell cycle stage. For example, XBP1U controls cellular proliferation by acting as a negative regulator of p53/p21, likewise IRE1/Xbp1 arm aids in cell cycle progression in T-Helper cells [170,171]. This forms another example of temporal regulation where the cell phase is determining IRE1 effector functions. It also reflects the harmony between the cell cycle and UPR that the UPR pathway is quite flexible to act with respect to the cellular state and each cell can respond differentially to the stress.

These aspects of IRE1 signaling open a new spectrum of knowledge about its regulation and signaling mechanism. Although the above-mentioned studies give a handful of evidence, the subject is still in infancy. A lot needs to be studied in this respect, which would introduce new horizons of knowledge about the IRE1 signaling. So far, we have been quite familiar with the intricacy in the mechanism of IRE1 operation and the dynamics of its functions. But weighing IRE1 in different spatiotemporal phases would not only serve to understand the complex

mechanisms associated with the protein but also decipher its role in various pathophysiological states.

7. Conclusion

Since the discovery of IRE1 as a signaling molecule responsible for the endoribonuclease activity involved in UPR, our knowledge about its structure and functions has increased tremendously. The mutational and crystallographic studies gave detailed insights of the IRE1 structure present in yeast and mammals. However, there remains inconsistency in the models of activation and the identification of precise factors involved. This discrepancy has aroused mainly because these activation models of IRE1 are purely based on structural studies, which undermines the role of protein factors that associate with IRE1 in a stress-dependent manner. The integration of structural and functional studies of IRE1 can yield a novel way to decipher the underlying mechanisms involved in its activation.

IRE1 is known for its divergent roles and peculiar functions in cells. Its ribonuclease outputs of Xbp1 splicing and RIDD are capable of operating circumstantially, giving rise to diverse cellular outcomes. Thus, it is imperative to have an in-depth understanding of the mechanisms involved in its RNase activity. There is an emerging consensus that the regulation of IRE1 operates dynamically. IRE1 regulation can be explained by an exquisite concept presented in the UPRosome. The UPRosome is a dynamic signaling platform in which many regulatory and adaptor proteins interact to activate and modulate downstream cascade of IRE1, and their association is dependent mainly on ER stress. Therefore, it seems that the expression pattern of IRE1 cofactors may help in determining the threshold of stress needed to engage downstream responses in different cell types. In conclusion, IRE1 emerges as a hotspot for ER stress studies, accounting for its unique yet discrete functions among which cell fate determination is of major significance.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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