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Forum Review

Unconventional Splicing of XBP-1 mRNA in the Unfolded Protein Response

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ABSTRACT

Cytoplasmic splicing is one of the major regulatory mechanisms of the unfolded protein response (UPR). The molecular mechanism of cytoplasmic splicing is unique and completely different from that of conventional nuclear splicing. The mammalian substrate of cytoplasmic splicing is XBP1 pre-mRNA, which is converted to spliced mRNA in response to UPR, leading to the production of an active transcription factor [pXBP1(S)] responsible for UPR. Interestingly, XBP1 pre-mRNA is also translated into a functional protein [pXBP1(U)] that negatively regulates the UPR. Thus, mammalian cells can quickly adapt to a change in conditions in the endoplasmic reticulum by switching proteins encoded in the mRNA from a negative regulator to an activator. This elaborate system contributes to various cellular functions, including plasma cell differentiation, viral infections, and carcinogenesis. In this short review, I briefly summarize research on cytoplasmic splicing and focus on current hot topics. *Antioxid. Redox Signal.* 9, 2323–2333.

INTRODUCTION

The splicing of XBP1 and HAC1 mRNAs is a central mechanism of the IRE1 pathway, one branch of the unfolded protein response (UPR) (5, 57, 66, 123, 127). Surprisingly, the splicing is catalyzed by unconventional splicing machinery and is thought to occur in the cytoplasm. In this short review, I describe (a) how cytoplasmic splicing was discovered, (b) the molecular mechanism, and (c) the biologic significance of cytoplasmic splicing.

CYTOPLASMIC SPLICING IN YEAST UPR

The discovery of HAC1 splicing

The study of cytoplasmic splicing started when IRE1 was cloned as a regulator of the unfolded protein response in *Saccharomyces cerevisiae* (13, 62). IRE1 encodes a transmembrane protein, Ire1p, that is located in the endoplasmic reticulum (ER) and contains a serine/threonine kinase domain (Fig. 1A). It had been speculated that Ire1p functions as a receptor-type protein kinase that transmits signals by phosphorylating downstream signaling molecules. But this was not the case. The discovery of HAC1, the downstream target of IRE, revealed that Ire1p contains an RNase domain that is similar to mammalian RNase L and converts HAC1 pre-mRNA into mature mRNA by splicing on the UPR (14, 17, 41, 63). Hac1p, a basic leucine zipper transcription factor translated from mature HAC1 mRNA, binds to a cis-acting element called UPRE (44, 61, 64) and activates the transcription of target genes with co-activators such as ADA5 (113). Targets of Hac1p include ER chaperones, including LHS1 and SCJ1, components of the ER-associated degradation (ERAD) machinery, such as DER1, HRD1 and UBC7, and factors involved in phospholipids biosynthesis (EPT1, INP51 and LPP1), translocation (SEC61, SLS1, and SEC62), and vesicular transport (ERV25, SEC13, and SEC24) (15, 70, 107). Interestingly, it was reported that Hac1p nega-

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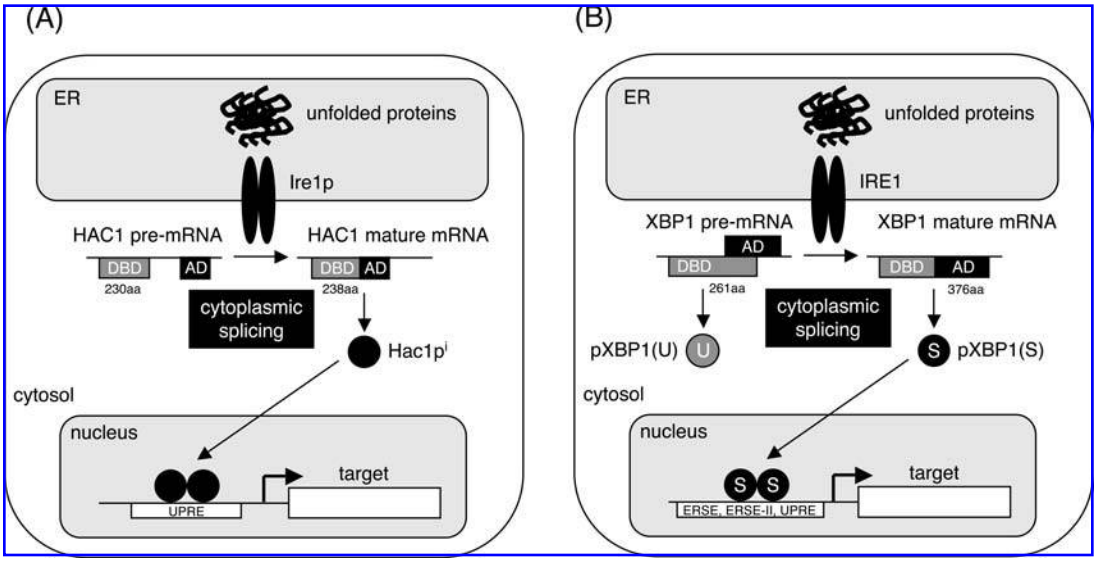

FIG. 1. Unconventional splicing of HAC1 and XBP1 pre-mRNAs. (**A**) The splicing of HAC1. In normal conditions, HAC1 pre-mRNA is not translated because an intron inhibits translation. When Ire1p is activated by accumulation of unfolded proteins, HAC1 pre-mRNA is converted to mature mRNA by unconventional splicing, leading to production of Hac1p^{i} that contains both the DNA-binding domain (DBD) and the activation domain (AD), and activates transcription of targets through a cis-element, UPRE. (**B**) The splicing of XBP1. In response to the UPR, XBP1 pre-mRNA is spliced by unconventional splicing mediated by IRE1. Then pXBP1(S) is translated from mature XBP1 mRNA and activates transcription of targets through cis elements such as ERSE, ERSE-II, and mammalian UPRE. Interestingly, XBP1 pre-mRNA is efficiently translated, leading to production of pXBP1(U).

tively regulates the differentiation of yeast cells in response to nitrogen starvation with the RPD3-SIN3 histone deacetylase complex (91).

The splicing of HAC1 mRNA is unconventional (Fig. 2). Conventional splicing is catalyzed by the spliceosome, which consists of multiple proteins and small nuclear RNAs (103). The two nucleotides at the 5′ and 3′ termini of an intron in conventional splicing are usually GU and AG (sometimes AU and AC), respectively, a rule known as Chambon's rule (6). The splicing reaction is sequential: the 5′ splice site is cleaved first,

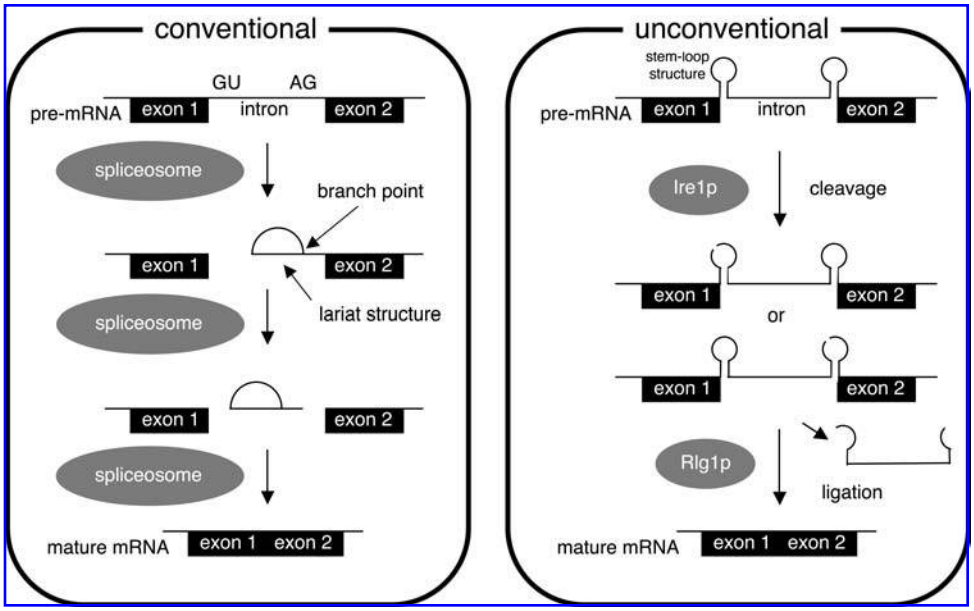

FIG. 2. Comparison of molecular mechanisms of conventional and unconventional splicing. (*Left*) Conventional splicing is catalyzed by the spliceosome (103), and the cleavage reaction proceeds sequentially (103). The nucleotide sequence at the exon–intron border complies with Chambon's rule (GU-AG rule) (6). (*Right*) Unconventional splicing of HAC1 is catalyzed by Ire1p and Rlg1p, and the order of cleavage of the exon–intron junctions is not predetermined. A pair of stem–loop structures exists at the cleavage sites (shown in Fig. 3).

and after the lariat structure has formed, the 3′ splice site is cleaved (89). In contrast, HAC1 pre-mRNA is cleaved by Ire1p, and ligated by a tRNA ligase, Rlg1p, and this process is independent of the spliceosome (96). At the exon–intron boundary is a pair of characteristic stem–loop structures that is recognized by Ire1p (Fig. 3A), instead of a consensus sequence such as GU-AG (42, 97). Moreover, the order of cleavage of the exon–intron junctions is not predetermined in HAC1 pre-mRNA.

The present understanding of the splicing reaction for HAC1 mRNA is as follows: when unfolded proteins accumulate in the ER, Ire1p dimerizes and is activated by autophosphorylation through its kinase domain (43, 76, 77, 79, 93, 111). The RNase domain of the activated Ire1p recognizes a CNGNNG motif in the stem–loop structures and cleaves HAC1 pre-mRNA at a specific position (see Fig. 3), releasing the intron and generating a 2′,3′-cyclic phosphate at the 3′ terminus of the 5′ exon and a free 5′-OH group at the 5′ terminus of the 3′ exon, respectively (Fig. 4). Rlg1p phosphorylates the 5′ end of the 3′ exon, cleaves the 2′,3′-cyclic phosphate at the 2′-position, adenylates the 5′ end of the 3′ exon, and then ligates the two exons. On ligation, AMP is released, and 2′-phosphate at the splice junction is removed, possibly by an NAD-dependent 2′-phosphotransferase (21). The phosphate of the activated Ire1p is removed by serine/threonine phosphatase Ptc2p, leading to inactivation of Ire1p (112).

The most important aspect of HAC1 splicing is where the reaction occurs. In most cases, conventional mRNA splicing occurs in the nucleus, because that is where the spliceosome is located, and pre-mRNA cannot be transported to the cytoplasm without being spliced (in specific cases, conventional splicing occurs in the cytoplasm: see later). In contrast, the splicing reaction of HAC1 pre-mRNA is thought to occur in the cytoplasm, because HAC1 pre-mRNA is transported to the cytoplasm (8), and the HAC1 pre-mRNA associated with polysomes is spliced on the UPR (88). Moreover, Rlg1p seems to be located in the cytoplasm, because tRNA splicing is thought to take place in the cytoplasm because components of tRNA splicing endonuclease, such as Sen2p and Sen54p, are present on the mitochondrial surface, and tRNA shuttles between the cytoplasm and the nucleus (101, 124). Indeed, Weissman and colleagues (28) showed that Rlg1p is located in the cytoplasm, although previously it was reported to be present in the nucleus (11). Although some reports suggest the possibility that the splicing of HAC1 pre-mRNA may occur in the nucleus as well as the cytoplasm (20, 41), currently available evidence supports the notion that HAC1 splicing occurs in the cytoplasm.

Consequence of HAC1 splicing

The splicing of HAC1 pre-mRNA is a key mechanism of the IRE1 pathway that allows production of an active transcription factor Hac1p, but the consequence of the splicing is still controversial. One possibility is that splicing allows the translation of HAC1 mRNA, because the intron of HAC1 pre-mRNA prevents its translation either by base pairing with the 5′ untranslated region (8, 88) (Fig. 5), or by inhibiting the transport of HAC1 pre-mRNA (41).

Another possibility, which is not mutually exclusive with that mentioned earlier, is that splicing changes the character of proteins encoded in HAC1 mRNA, because removal of the intron replaces the C-terminal tail of Hac1p with a different peptide. As shown in Fig. 1A, HAC1 pre-mRNA and mature mRNA encode Hac1p^{u} (230 amino acid residues) and Hac1p^{i} (238 amino acid residues), respectively. It is reported that Hac1p^{u} is more susceptible to degradation by the proteasome (14), and that Hac1p^{i} is more transcriptionally active, as it contains a transcriptional activation domain (65).

CYTOPLASMIC SPLICING IN MAMMALIAN UPR

The discovery of XBP1 splicing

Elucidation of the yeast IRE1-HAC1 system prompted the search for a mammalian counterpart. Two Ire1p homologs, IRE1α and IRE1β, were identified in mammalian cells (30, 104, 105, 109). However, an attempt to isolate a Hac1p homologue failed because mammalian genomes contain no HAC1 homologue. By contrast, it was revealed that mammalian IRE1 functions other than as a catalyst of unconventional splicing, that is, in the cleavage of rRNA (30) and transmission of apoptotic signals in association with TRAF2 (108). Moreover, the ATF6 pathway, another signaling system independent of unconventional splicing, was found to regulate UPR-specific transcriptional induction (23, 24, 116, 117, 119, 121). Thus, it had been speculated that mammalian cells do not have the unconventional splicing system. But again, this was not the case.

Based on extensive analyses of mammalian and nematode UPR, three groups eventually identified XBP1 as a substrate of mammalian IRE1 (7, 54, 94, 118). Because the DNA-binding domain (DBD) and the transcriptional activation domain (AD) are encoded in separate open reading frames (ORFs) in the XBP1 pre-mRNA, pXBP1(U), a protein translated from XBP1 pre-mRNA, has only the DBD and cannot activate transcrip-

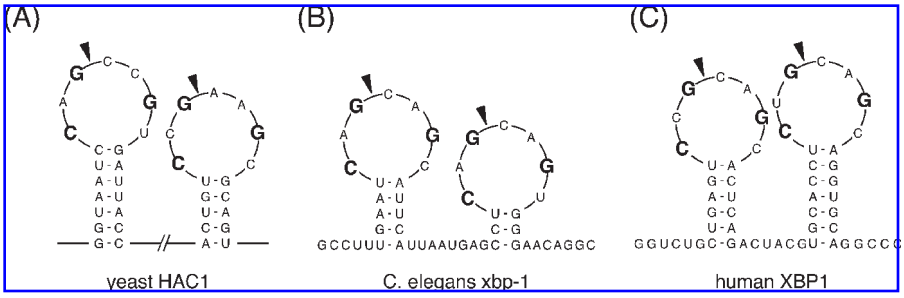

FIG. 3. Stem–loop structures found in budding yeast HAC1, *Caenorhabditis elegans* xbp-1, and human XBP1 mRNAs. The cleavage sites and conserved CNGNNG motifs are indicated by arrowheads and bold letters, respectively.

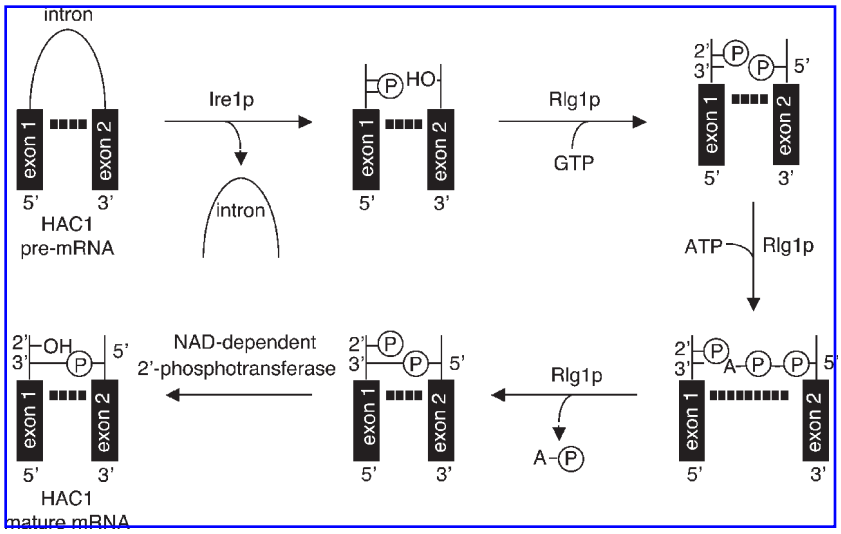

FIG. 4. Precise reaction mechanism of the splicing of HAC1 pre-mRNA. Because the interaction through base pairings (*thick dotted line*) connects two exons of HAC1 mRNA, they do not separate after cleavage by Ire1p. Rlg1p catalyzes a series of complicated reactions that is also observed in tRNA processing. The final reaction is thought to be mediated by NAD-dependent 2′-phosphodiesterase, as in the case of tRNA processing.

tion (51, 118) (see Fig. 1B). In response to UPR, XBP1 pre-mRNA is spliced by IRE1 and converted to mature mRNA, from which an active transcription factor, pXBP1(S), containing both the DBD and AD, is translated, because removal of an intron joins the two ORFs. It binds to a cis-acting element ERSE, ERSE-II, and mammalian UPRE (115, 119, 120), and activates transcription of target genes. Targets of XBP1 include ER chaperones (BiP, ERdj4, ERdj5, HEDJ, GRP58, and PDI-P5), ERAD components (EDEM, OS9, Herp, and $p58^{IPK}$), transcription factors (CHOP and XBP1), and components of secretory pathways (SEC23B, SEC24C, SEC61A, SEC61G, SRP54, and TRAM1) (52, 75, 92, 120, 121).

The nucleotide sequence at the exon–intron border of XBP1 pre-mRNA does not comply with Chambon's rule, and instead a pair of stem–loop structures is observed, similar to that in HAC1 pre-mRNA. The CNGNNG motif in the stem–loop structures is conserved in XBP1 pre-mRNA, and this motif is essential for recognition by IRE1 (see Fig. 3C). These results indicate that the splicing of XBP1 is unconventional, like that of HAC1, and that the mechanism of unconventional splicing is conserved from yeast to mammals.

XBP1 encodes a basic leucine zipper–type transcription factor, but is not an HAC1 homologue because pXBP1(S) shows no homology to $Hac1p^{i}$, other than the DBD. In addition, the intron of XBP1 pre-mRNA is very short (26nt), as compared with that of HAC1 pre-mRNA (252nt), and does not inhibit translation of XBP1 pre-mRNA. Actually, pXBP1(U) is efficiently produced in the absence of unfolded protein.

What is the function of pXBP1(U), a protein encoded in XBP1 pre-mRNA? Recent studies found that the expression of pXBP1(U) is increased during the recovery phase of UPR, and that pXBP1(U) binds to pXBP1(S), sequesters it from the nucleus, and enhances its degradation by the proteasome, leading to a complete shut-off of UPR-induced transcription (106, 122) (Fig. 6). These findings suggest that pXBP1(U) is a negative-feedback regulator of mammalian UPR. Thus, XBP1 mRNA encodes negative and positive regulators in unspliced and spliced mRNA, respectively. Cytoplasmic splicing can rapidly switch these two forms in response to a change in conditions in the ER without *de novo* transcription, because it can splice mRNA that already exists in the cytoplasm, where translation is occurring. Moreover, because unspliced mRNA is converted to mature mRNA and disappears, unnecessary translation of unspliced mRNA is prevented. Thus, cytoplasmic splicing is a quick, energy-efficient, and garbage-free mechanism.

Is the splicing of XBP1 actually cytoplasmic splicing?

Several lines of evidence strongly support the notion that the splicing of XBP1 takes place in the cytoplasm. First, XBP1 pre-mRNA is transported to the cytoplasm and translated (122). In-

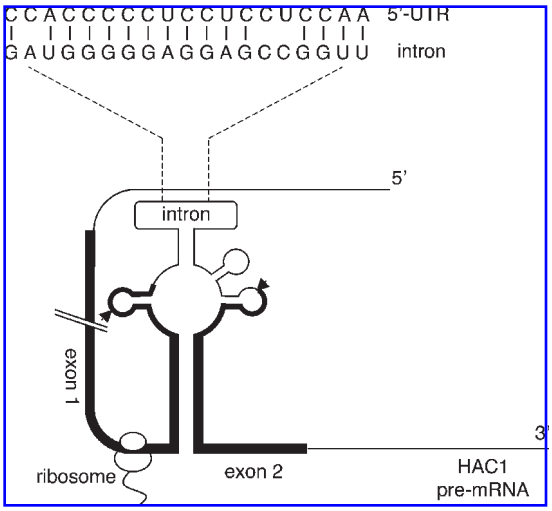

FIG. 5. Molecular mechanism for translational blockade of HAC1 pre-mRNA. The 5′-untranslated region (UTR) forms base pairings with the intron that stalls ribosomes and inhibits translation of HAC1 pre-mRNA. Thus, removal of the intron by Ire1p on the UPR induces translation of $Hac1p^{i}$. Exons and cleavage sites are shown by *thick lines* and *arrowheads*, respectively.

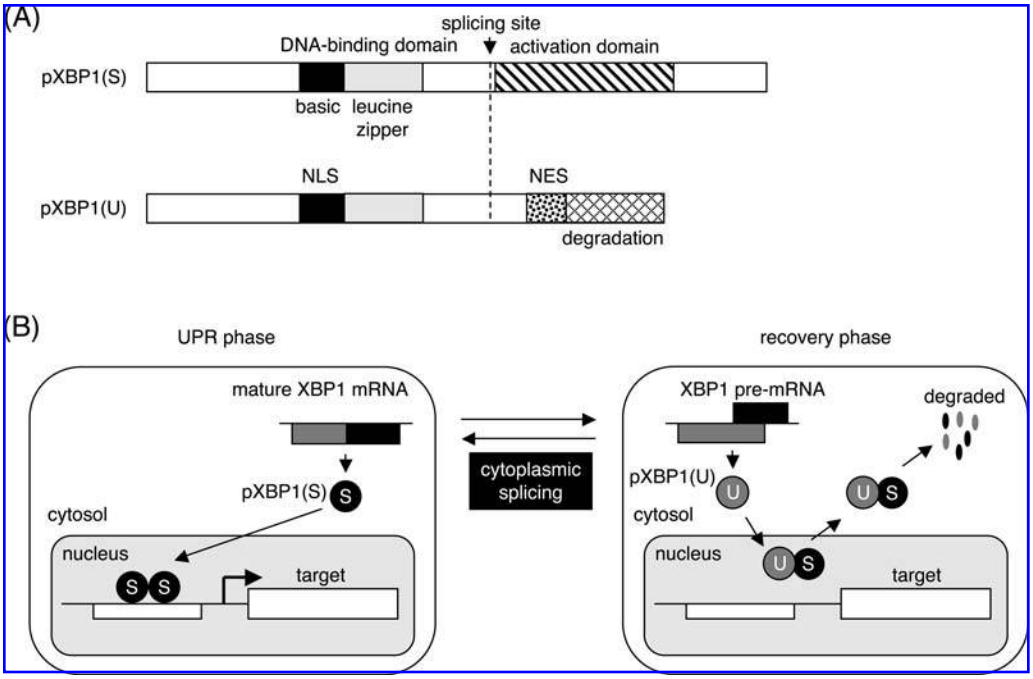

FIG. 6. Feedback regulation of mammalian UPR by pXBP1(U). (**A**) The modular structures of pXBP1(U) and pXBP1(S). Both pXBP1(U) and pXBP1(S) have a DNA-binding domain that consists of a basic region and a leucine zipper region. The basic region also functions as a nuclear localization signal (NLS). pXBP1(S) contains the transcriptional activation domain that activates transcription of target genes. Conversely, pXBP1(U) has a nuclear exclusion signal (NES), as well as NLS, and shuttles between the nucleus and cytosol. Moreover, pXBP1(U) contains the degradation domain and is rapidly degraded by the proteasome. (**B**) Dynamic interplay between pXBP1(U) and pXBP1(S). During the UPR phase, pXBP1(S) and other UPR-transcription factors induce expression of targets, including ER chaperones and ERAD components, that enhance the recovery from the UPR. At the recovery phase, newly transcribed XBP1 mRNA remains unspliced because IRE1 is inactivated, leading to production of pXBP1(U). pXBP1(U) shuttles between the nucleus and cytosol, binds to pXBP1(S) through the leucine zipper region, sequesters pXBP1(S) by the function of the NES, and enhances the degradation of pXBP1(S) by the proteasome.

terestingly, a recent report suggests that XBP1 mRNA is abundant on ER-bound ribosomes (100). Second, the RNase domain of IRE1 seems to reside in the cytoplasm, judging from its amino acid sequence, because it contains both a signal sequence and one transmembrane domain, suggesting that IRE1 is a type I transmembrane protein (104). Third, XBP1 pre-mRNA delivered directly to the cytoplasm by T7 polymerase-mediated transcription or RNA transfection, can be spliced on UPR (3). Fourth, the RNase domain of IRE1 expressed in the cytoplasm can splice XBP1 pre-mRNA (3, 31).

Conversely, several reports suggest the possibility that XBP1 splicing may also occur in the nucleus as well as the cytoplasm. First, it is reported that IRE1 is located predominantly in the nuclear envelope, especially in the nuclear pore complex (104), and that the RNase domain released after cleavage of IRE1 on UPR translocates into the nucleus (72), although the biologic significance of nuclear (or nuclear membrane) localization of IRE1 has not been clarified. Second, mammalian tRNA splicing is thought to occur in the nucleus (82), suggesting that the splicing of XBP1 may occur in the nucleus if tRNA ligase joins XBP1 pre-mRNA cleaved by IRE1.

However, it is highly controversial that tRNA that the mechanism of tRNA splicing is conserved from yeast to mammals. No Rlg1p homologue is found in mammalian genomes, and mammalian tRNA ligation has been thought to occur without formation of a 2′ phosphate, strongly suggesting that the ligation steps of tRNA splicing in yeast and mammals proceed by fundamentally different mechanisms (1, 25, 50, 90). If this is the case, it is likely that the RNA ligase for XBP1 splicing is not identical to the mammalian tRNA ligase, that the precise mechanism of XBP1 splicing is different from that of HAC1 splicing, and that the splicing of XBP1 pre-mRNA occurs in the cytoplasm. Conversely, it was also reported that a yeast tRNA-like activity can be detected in mammalian cells (128), suggesting that mammalian tRNA ligation is conventional. In plants, a yeast tRNA-like activity has been reported, but plant tRNA ligase shows no homology to Rlg1p (18). Interestingly, a yeast tRNA-like activity (2′ phosphotransferase activity) was observed in *Escherichia coli*, and the *E. coli* enzyme can substitute the yeast one *in vivo* (99), suggesting that this enzyme has a second function other than tRNA ligation. Taken as a whole, identification and characterization of components of mammalian splicing machinery, including the RNA ligase, will finally demonstrate that the splicing of XBP1 occurs in the cytoplasm and elucidate the precise molecular mechanism of mammalian unconventional splicing.

BIOLOGIC FUNCTION OF XBP1

Recent studies revealed that XBP1 and its cytoplasmic splicing are involved in various biologic phenomena. In this section, I briefly summarize these phenomena and underlying mechanisms.

XBP1 and antibody production

XBP1 (X box–binding protein 1) was first identified as a transcription factor that binds to a cis-acting element of MHC class II genes (55). Subsequent studies suggest that XBP1 is involved in the immune response, especially in the B-cell lineage (12, 56, 71, 78, 83). Then it was demonstrated that XBP1 and its splicing are essential for plasma cell differentiation and antibody production (22, 29, 68, 86, 106). Plasma cells have an enormously extended ER structure to support the robust production of immunoglobulin proteins, which probably induces UPR. Thus, it is quite reasonable that XBP1 is indispensable for plasma cell differentiation. XBP1 is also required for the expanded ER structure in exocrine cells, such as pancreatic and salivary gland acinar cells (53), suggesting that cytoplasmic splicing of XBP1 is a regulatory system essential for the biogenesis of the secretory machinery that vigorously produces secretory proteins.

XBP1 and lipid synthesis

The differentiation of exocrine cells accompanies a robust expansion of the ER structure to accommodate large amounts of nascent secretory proteins, as mentioned earlier. Interestingly, ectopic expression of pXBP1(S) induces expansion of the ER (92, 98), suggesting that XBP1 is involved in lipid synthesis, although microarray analyses of the XBP1 target genes could not identify genes involved. Interestingly, enforced expression of pXBP1(S) enhances the activity of the enzymes responsible for phosphatidylcholine synthesis and induces synthesis of phosphatidylcholine (98), suggesting that XBP1 regulates lipid synthesis posttranscriptionally or posttranslationally or both.

XBP1 and viral infections

XBP1 is identical to TREB5 (TRE-binding protein 5), isolated as a transcription factor binding to the tax-responsive enhancer (TRE) of HTLV (59, 125), suggesting a link between XBP1 and viral infections. Because large amounts of viral proteins are synthesized during viral infections, it is rational that a viral infection induces UPR and the splicing of XBP1. Actually, infections of hepatitis C virus, Flavivirus, and Borna activate XBP1 splicing (4, 27, 114, 126). Interestingly, it was reported that the S promoter of hepatitis B virus is activated by pXBP1(S) (27), and that an infection with hepatitis C virus suppresses XBP1 splicing (102), suggesting a complex relation between viral infections and XBP1. Because the RNase domain of IRE1 and kinase domain of PERK are similar to RNase L and PKR, respectively, which are involved in defense against viral infections, the UPR and antiviral system may share a common ancestral mechanism. Thus, it may be possible to develop efficient antiviral medicines by elucidating the close relation between UPR and viruses.

XBP1 and liver

Because hepatocytes also produce large amounts of secretory proteins, it is reasonable that the splicing of XBP1 is indispensable for the homeostasis of hepatocytes. A lack of XBP1 is embryonic lethal in mice, with abnormalities such as growth retardation and pale coloration observed from 12.5 to 13.5d (85). The most notable defect is hypoplastic fetal liver. One of specific target genes of XBP1 in hepatocytes is α-fetoprotein, which may be a regulator of hepatocyte growth. In XBP1$^{-/-}$ mice, reduced transcriptional induction of the α-fetoprotein gene results in apoptosis of hepatocytes, leading to reduced hematopoiesis and finally death from severe anemia. XBP1$^{-/-}$ mice also show cellular necrosis in cardiac myocytes at the embryonic stage, suggesting that XBP1 is essential for cardiogenesis, although the underlying mechanism has not been clarified (58).

XBP1 and cancer

XBP1 is identical to HTF (hepatocarcinogenesis-related transcription factor) originally isolated as a gene product whose expression is enhanced in hepatocellular carcinomas (46), suggesting a relation between XBP1 and carcinogenesis. Actually, the splicing of XBP1 is activated in hepatocellular carcinoma and breast cancer (49, 95). Interestingly, BiP, one of the targets of XBP1, was also identified as a protein whose expression is increased in tumors (84). It is speculated that the inside of a solid tumor is hypoxic, which induces UPR and XBP1 splicing (87). Because XBP1 is protective against hypoxia, it may be a useful anticancer target (47).

XBP1 and mental disorders

From the study of twins discordant with respect to bipolar disorder, XBP1 was identified as a genetic risk factor for the disease (26, 33, 36–40, 60), although the conclusion is still disputed (10). A polymorphism (−116C to G) was found in the promoter region of the XBP1 gene, which may be important for positive-feedback regulation by XBP1.

XBP1 is also involved in schizophrenia. A case–control study suggested that the functional polymorphisms −197C/G and −116C/G in XBP1 are linked to schizophrenia (9, 32, 34, 39, 67, 110). XBP1 is also closely associated with neuronal functions, such as brain trauma (2, 35, 48, 67, 80, 81), although the underlying mechanism remains to be elucidated.

OTHER SUBSTRATES OF CYTOPLASMIC SPLICING

At present, the only known substrates of cytoplasmic splicing are HAC1 and XBP1 pre-mRNAs. Because it is highly possible that others exist, I describe current information about potential substrates in this section.

Other substrates of IRE1 in eukaryotes

The fact that yeast cells have HAC1 and mammalian cells have XBP1 raises the question of when XBP1 evolved during

evolution. *In silico* analyses of nucleotide databases suggest that XBP1 is conserved among mammals, birds, amphibians, fish, the fruit fly, and nematodes (see Fig. 3B) (69, 94). Introns of these animals are very short (vertebrates, 26nt; fly, 53nt; nematode, 24nt), suggesting that translation of pre-mRNAs is not inhibited. As for IRE1, it was found in a much wider range of organisms, such as *Schizosaccharomyces pombe* and flowering plants (45, 74). However, neither an HAC1 nor an XBP1 homologue has been identified in fission yeast and plants, suggesting that a third class of IRE1 substrates may exist in these organisms.

It is possible that additional IRE1 substrates exist in budding yeast and mammalian cells. Surprisingly, the *in silico* analyses revealed that HAC1 and XBP1 are the only substrates of IRE1 containing a stem–loop structure (69, 73), although this finding does not exclude the possibility that IRE1 substrates can be identified experimentally.

Other cytoplasmic splicing systems

Conventional splicing using the spliceosome takes place in the cytoplasm, with two exceptions. The first exception is the splicing that occurs in platelets. As platelets have no nucleus, conventional splicing actively occurs in the cytoplasm, which contains essential spliceosome factors including small nuclear RNAs, splicing proteins, and accumulated pre-mRNAs (16). Interestingly, interleukin-1β pre-mRNA is spliced in response to integrin engagement and surface receptor activation in the platelet, leading to the production of the cytokine. The second exception is the RNA splicing found in neuronal dendrites (19). The cytoplasm of dendrites (dendroplasm) contains functional components of the conventional splicing machinery, which can efficiently splice pre-mRNA. The functional significance of this "dendrite splicing" is unclear, but it may be convenient for splicing to occur at the dendrites as they are located far from the nucleus. Cells may generate a diversity of postsynaptic responses that increase the molecular complexity and functional capacity of the synapse. These two systems of cytoplasmic splicing are catalyzed by the machinery of conventional splicing located in the cytoplasm. It is also possible that unconventional splicing systems exist other than the IRE1 system.

CONCLUSIONS AND PERSPECTIVES

Research into cytoplasmic splicing has just branched out from the mainstream study of UPR, and the mechanistic details of mammalian cytoplasmic splicing remain elusive. As this field of research expands, the biologic significance of cytoplasmic splicing will be clarified further, and scientific achievements will be translated into clinical applications for the treatment of neurodegenerating diseases, viral infections, and cancer.

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ABBREVIATIONS

AD, activation domain; AMP, adenosine monophosphate; ATF6, activating transcription factor 6; NAD, nicotinamide adenine dinucleotide; BiP, binding protein; CHOP, C/EBP-homologous protein; DBD, DNA-binding domain; EDEM, ER degradation enhancing a -mannosidase-like protein; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERdj4, ER dnaJ 4; HEDJ, human ER-associated dnaJ; ERSE, ER stress-response element; GRP58, glucose-regulated protein 58 kDa; HAC1, homologous to ATF/CREB 1; Hac1p, HAC1 protein; $Hac1p^{U}$, uninduced form of HAC1 protein; $Hac1p^{i}$, induced form of HAC1 protein; Herp, homocysteine-inducible, endoplasmic reticulum stress-inducible protein; HTF, hepatocarcinogenesis-related transcription factor; HTLV, human T-cell leukemia virus; IRE1, inositol requirement 1; Ire1p, inositol requirement 1 protein; nt, nucleotide; ORF, open reading frame; OS9, osteosarcoma 9; $p58^{IPK}$, 58-kDa inhibitor of protein kinase; PDI-P5, protein disulfide isomerase P5; PERK, PRKR-like endoplasmic reticulum kinase; PEST, praline-, glutamate-, serine-, and threonine-rich region; PKR, double-stranded RNA-dependent protein kinase; pre-mRNA, precursor mRNA; Ptc2p, type 2C protein phosphatase; pXBP1(U), unspliced form of XBP1 protein; pXBP1(S), spliced form of XBP1 protein; Sen2p, splicing endonuclease 2 protein; SRP54, signal-recognition particle subunit 54; TRAF2, TNF receptor–associated factor 2; TRAM1, translocating chain–associating membrane protein 1; TRE, tax-responsive enhancer; TREB5, tax-responsive enhancer binding protein 5; XBP1, X box–binding protein; UPR, unfolded protein response; UPRE, unfolded protein response element.

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