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## IRE1, a double-edged sword in pre-miRNA slicing and cell death

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### Abstract

IRE1 $\alpha$ , the most conserved transducer of the unfolded protein response, plays critical roles in many biological processes and cell fate decisions. Reporting in *Science*, Upton et al. (2012) broadened our understanding of IRE1 $\alpha$  as a cell-death executioner, showing that upon ER stress, IRE1 $\alpha$  degrades microRNAs to promote translation of caspase-2.

In eukaryotic cells, the endoplasmic reticulum (ER) is a highly specialized organelle responsible for the translation, folding, and modification of approximately one-third of the cell's proteome. Upon accumulation of unfolded/misfolded proteins in the ER, cells activate the unfolded protein response (UPR) that, in metazoans, is initiated by three ER transmembrane protein sensor(s) in metazoans: inositol requiring enzyme 1 alpha (IRE1 $\alpha$ ), PKR-like ER kinase (PERK), and activated transcription factor 6 alpha (ATF6 $\alpha$ ). The UPR is essential for normal cellular and organismal physiology and contributes to the etiology of many diseases (Wang and Kaufman, 2012). Although initial UPR activation provides an adaptive response, severe or chronic UPR activation re-directs the adaptive response into a pro-apoptotic response, although the mechanisms are unknown. Among the ER stress sensors, IRE1 $\alpha$  is conserved from yeast to humans. IRE1 $\alpha$  has both protein kinase and endoribonuclease (RNase) activities that were originally characterized to initiate removal of a 26 base intron from X-box binding protein 1 (*Xbp1*) mRNA, thereby producing an active transcription factor that induces genes encoding adaptive functions to limit protein misfolding in the ER. However, IRE1 $\alpha$  has a growing list of additional mRNA cleavage substrates identified through regulated IRE1-dependent degradation (RIDD) of mRNAs (Han et al., 2009; Hollien et al., 2009). In a recent report in *Science*, Upton *et al.* showed that IRE1 $\alpha$  cleaves a new class of RNAs: microRNAs (miRs) that repress translation through binding to sequences in the 3' end of mRNAs. IRE1 $\alpha$ -mediated cleavage of miRs releases a translational block on Caspase 2 (*Casp2*) mRNA (Upton et al., 2012). Increased expression of *Casp2* mRNA then contributes to apoptotic cell death through proteolytic cleavage of Bid, which causes cytochrome c release from mitochondria.

Upton et al. (2012) first demonstrated that treatment with brefeldin A, which causes protein accumulation in the ER, increases CASP2 protein expression in wild-type and *Xbp1*<sup>-/-</sup>, *Perk*<sup>-/-</sup>, and *Atf6a*<sup>-/-</sup> mouse embryo fibroblasts (MEFs), but not in *Ire1a*<sup>-/-</sup> MEFs. Although

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there was no change in the total level of *Casp2* mRNA, polysome-associated *Casp2* mRNA increased in the wild-type MEFs, but not in the *Ire1 $\alpha$* <sup>-/-</sup> MEFs. Importantly, sustained activation of IRE1 $\alpha$  reduced levels of *miRs* -17, -34a, -96 and -125b, miRs that normally repress *Casp2* mRNA translation. An *in vitro* nuclease assay demonstrated that IRE1 $\alpha$  directly cleaves the *miR-17* precursor at three sites distinct from those cleaved by DICER. Perhaps most convincingly, transfection of anti-miRs, which protect the miRNAs from degradation by IRE1 $\alpha$  prevented *Casp2* mRNA translational derepression, as shown by western blotting. In addition, anti-*miR-17* expression was overcome by over-expression of IRE1 $\alpha$ . The authors further showed that proteolytic cleavage of Bid occurs downstream of IRE1 $\alpha$ -dependent *Casp2* mRNA translational derepression.

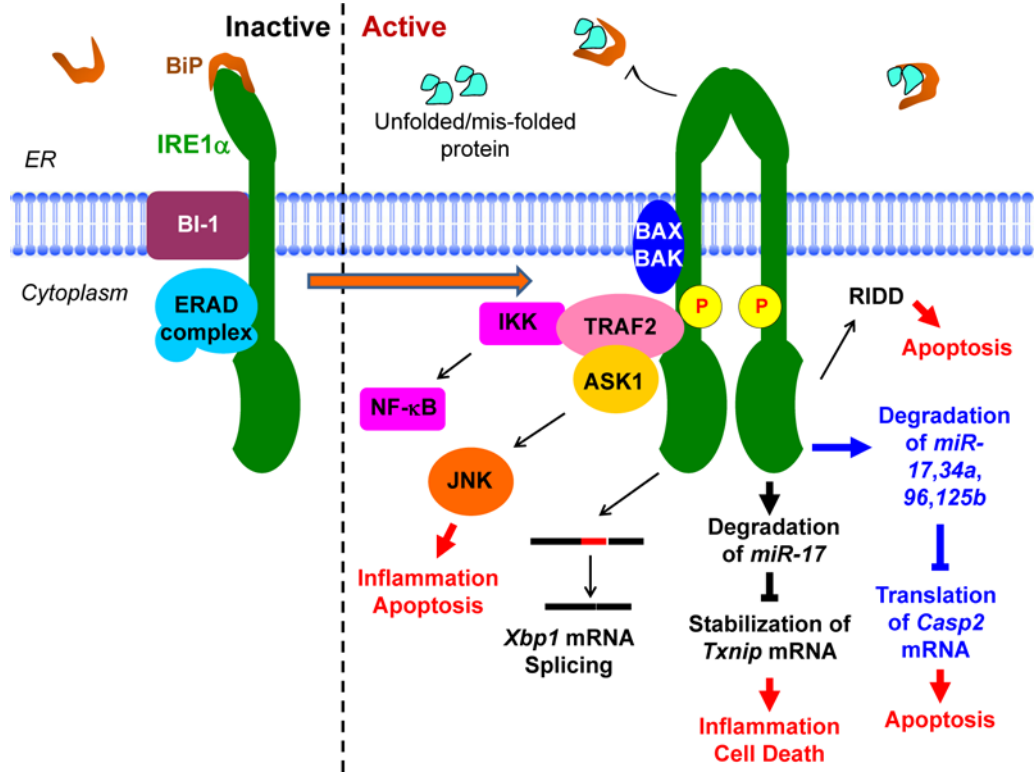
The findings from Upton et al. (2012) show that IRE1 $\alpha$  cleaves precursor miRs (pre-miR); an event that likely occurs in the nucleus or as the pre-miRs transit through the nuclear pore to the cytoplasm. Although IRE1 $\alpha$ -mediated *Xbp1* mRNA splicing occurs in the cytoplasm, IRE1 $\alpha$  is localized to the inner nuclear envelope (Lee et al., 2002), consistent with a function in nuclear RNA processing. The studies of Upton et al. (2012) provide one example by which IRE1 $\alpha$  activates apoptosis, but presumably there are others. Recently, PERK and IRE1 $\alpha$  signaling were shown to induce pro-oxidant TXNIP. Whereas PERK signaling induces ATF5 to activate *Txnip* transcription, IRE1 $\alpha$  nuclease cleaves *miR-17* to stabilize *Txnip* mRNA (Lerner et al., 2012; Osowski et al., 2012). As it is now evident that IRE1 $\alpha$  regulates miR production, there may be a multitude of processes that are regulated through IRE1 $\alpha$  that will be identified and characterized in the future.

The IRE1 $\alpha$ -dependent derepression of *Casp2* mRNA translation through miR cleavage was shown to occur in MEFs, mouse insulinoma, and human kidney cell lines. If this IRE1 $\alpha$ -dependent derepression of CASP2 occurs in additional cancerous and/or differentiated cell types that secrete high levels of protein, this pathway may be of greater physiological significance. In addition, chemical inhibitors of IRE1 $\alpha$  RNase activity now exist (Mimura et al., 2012) that should be tested for the potential to divert apoptosis in response to ER stress. Finally, although IRE1 $\alpha$  activation increases the expression of CASP2, there is another, yet unknown, signal that is required for its activation into a functional protease.

In summary, the authors have identified a pro-apoptotic pathway that emanates from IRE1 $\alpha$ . This IRE1 $\alpha$ -dependent pathway toward apoptosis adds to the other known IRE1 $\alpha$ -mediated pathways including *Xbp1* mRNA splicing, regulated IRE1-dependent decay (RIDD) of mRNAs, activation of the cJun N-terminal kinase (JNK), and nuclear factor kappa B (NF $\kappa$ B) pathways and inflammasome activation (Wang and Kaufman, 2012). Considering that the loss of IRE1 $\alpha$  and/or XBP1 signaling is detrimental, especially for professional secretory cells (which includes pancreatic  $\beta$  cells, plasma cells, hepatocytes, gastric zymogenic cells, and Paneth cells in the small intestine), it appears IRE1 $\alpha$  functions as a double-edged sword in the life versus death decision. The RIDD-dependent degradation of mRNAs by IRE1 $\alpha$  is proposed to protect cells by reducing the protein-folding burden on the ER. However, RIDD can also perform the role of cell executioner by degrading mRNAs encoding pro-survival proteins during prolonged ER stress. In addition, the IRE1 $\alpha$ -JNK pathway has been shown to cause apoptosis under some cellular stresses (Tabas and Ron, 2011). The findings of Upton et al. (2012) thus further our understanding of IRE1 $\alpha$  as a regulatory hub of the cell fate decision. CASP2 is the most evolutionarily conserved of caspases identified to date. Although its role in the apoptotic cascade is still elusive, CASP2 regulates NF $\kappa$ B signaling and functions as a tumor suppressor (Bouchier-Hayes and Green, 2012). Given the critical role of IRE1 in NF $\kappa$ B activation, inflammation, and tumorigenesis, it is important to determine how caspase-2 and its downstream targets contribute to these cellular processes during ER stress.

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**Figure 1. IRE1α-mediated signaling of life and death**

Dimerization of IRE1α induces its activation and initiates downstream signaling through the recruitment of TRAF2 and RNase activation to promote *Xbp1* mRNA splicing, degradation of mRNAs, and degradation of miRNAs.