conserved role in regulating transcriptional directionality.

These results suggest that formation of gene loops influence unidirectional transcription. How might this work? Based on the acetylation of histone H4 in promoters of genes that exhibit divergent SRTs, the authors postulate that looping leads to directional histone deacetylation and repression upstream of the promoter. An alternative view is that looping leads to directional acetylation within the loop. Also, because recruitment of RNAPII to the promoter is often rate limiting, if intragenic looping permits more efficient recycling of RNAPII for reinitiation, it is tempting to speculate that this might also bias transcriptional directionality. Many components of the preinitiation complex remain associated with the promoter, potentially serving as a scaffold to allow for such recycling. Consistent with this notion, RNAPII associated with the active *hsp70* locus in flies is not readily exchanged with the nuclear pool, suggesting that this locus is somehow "compartmentalized" and that RNAPII is recycled (Zobeck et al., 2010). Resolutions of these questions will await a better understanding of how looping affects chromatin structure, histone acetylation, and RNAPII function.

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IRE1, a Double-Edged Sword in Pre-miRNA Slicing and Cell Death

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IRE1α, 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 IRE1α as a cell-death executioner, showing that upon ER stress, IRE1α 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 is initiated by three ER transmembrane protein sensors: inositol requiring enzyme 1 alpha (IRE1α), PKR-like ER kinase (PERK), and activated transcription factor 6 alpha (ATF6α). 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 redirects the adaptive response into a proapoptotic response, although the mechanisms are unknown. Among the ER stress sensors, IRE1α is conserved from yeast to humans. IRE1α has both protein kinase and endoribonuclease (RNase) activities that, in metazoans, 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, IRE1α 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 IRE1α cleaves a new class of RNAs: microRNAs (miRs) that repress translation through binding to sequences in the 3′ end of mRNAs. IRE1α-mediated cleavage of miRs releases a translational

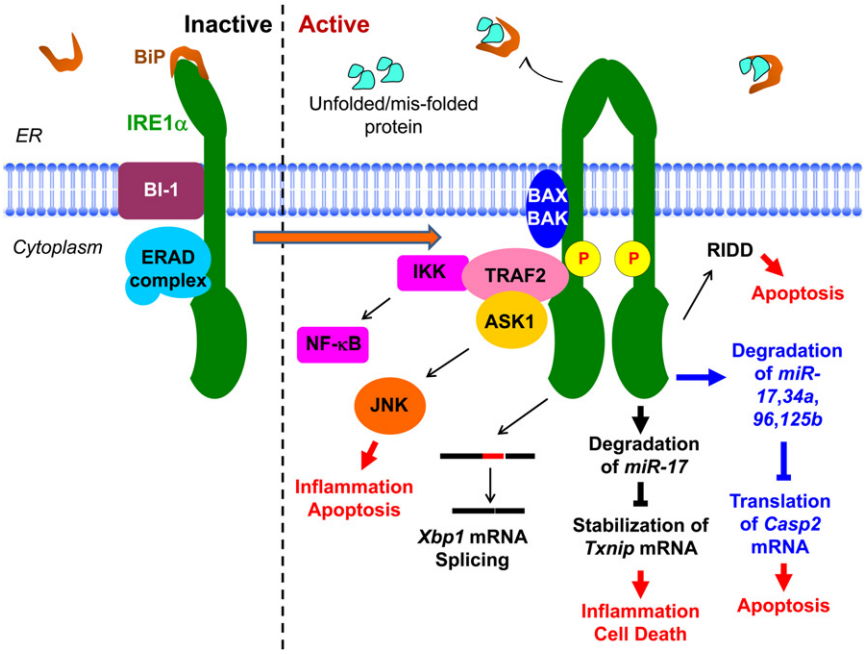

Figure 1. IRE1α-Mediated Signaling of Life and Death
Dimerization of IRE1α induces its activation and initiates downstream signaling through the recruitment of TRAF2 and RNase activation to promote *Xbp1* mRNA splicing, degradation of mRNAs, and degradation of miRs.

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*$^{-/-}$, *Perk*$^{-/-}$, and *Atf6α*$^{-/-}$ mouse embryo fibroblasts (MEFs), but not in *Ire1α*$^{-/-}$ MEFs. Although 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 *Ire1α*$^{-/-}$ MEFs. Importantly, sustained activation of IRE1α reduced levels of *miR-17*, *miR-34a*, *miR-96*, and *miR-125b*, miRs that normally repress *Casp2* mRNA translation. An in vitro nuclease assay demonstrated that IRE1α 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 miRs from degradation by IRE1α, prevented *Casp2* mRNA translational derepression, as shown by western blotting. In addition, anti-*miR-17* expression was overcome by overexpression of IRE1α. The authors further showed that proteolytic cleavage of Bid occurs downstream of IRE1α-dependent *Casp2* mRNA translational derepression.

The findings from Upton et al. (2012) show that IRE1α cleaves precursor miRs (pre-miRs), an event that likely occurs in the nucleus or as the pre-miRs transit through the nuclear pore to the cytoplasm. Although IRE1α-mediated *Xbp1* mRNA splicing occurs in the cytoplasm, IRE1α 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 IRE1α activates apoptosis, but presumably there are others. Recently, PERK and IRE1α signaling were shown to induce pro-oxidant TXNIP, leading to activation of the NLRP3 inflammasome and IL-1β expression. Whereas PERK signaling induces ATF5 to activate *Txnip* transcription, IRE1α RNase cleaves *miR-17* to stabilize *Txnip* mRNA (Lerner et al., 2012; Oslowski et al., 2012). Because it is now evident that IRE1α regulates miR production, there may be a multitude of processes that are regulated through IRE1α that will be identified and characterized in the future.

The IRE1α-dependent derepression of *Casp2* mRNA translation through miR cleavage was shown to occur in MEFs, mouse insulinoma, and human kidney cell lines. If this IRE1α-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 IRE1α 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 IRE1α 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 proapoptotic pathway that emanates from IRE1α. This IRE1α-dependent pathway toward apoptosis adds to the other known IRE1α-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 (NFκB) pathways and inflammasome activation (Wang and Kaufman, 2012) (Figure 1). Considering that the loss of IRE1α and/or XBP1 signaling is detrimental, especially for professional secretory cells (which includes pancreatic β cells, plasma cells, hepatocytes, gastric zymogenic cells, and Paneth cells in the small intestine), it appears IRE1α functions as a double-edged sword in the life-versus-death decision. The RIDD-dependent degradation of mRNAs by IRE1α is proposed to protect cells by reducing the protein-folding burden on the ER (Hollien et al., 2009). However, RIDD can also perform the role of cell executioner by degrading mRNAs encoding pro-survival proteins during prolonged ER stress (Han et al., 2009). In addition, the IRE1α-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 IRE1α 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 NFκB signaling and functions as a tumor suppressor (Bouchier-Hayes and Green, 2012). Given the critical role of IRE1

in NFκB 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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Choreographing the Axo-Dendritic Dance

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The assembly of neuronal synapses in the brain relies on a sophisticated bidirectional signal exchange between synaptic partners. In a recent issue of *Neuron*, Ito-Ishida and colleagues (2012) uncover a morphogenetic program underlying the formation of presynaptic terminals.

The formation of neuronal synapses during development of the central nervous system represents a remarkable morphogenetic process. Bidirectional communication between axons and dendrites instructs the assembly of an asymmetric synaptic junction (Shen and Scheiffele, 2010). Thus, dendrite-derived signals transform a segment of a thin axonal process into a varicosity filled with synaptic vesicles docked at sites for regulated neurotransmitter release. Simultaneously, axonal signals drive the accumulation of neurotransmitter receptors opposite these release sites. For most synapses secreting the transmitter glutamate, these accumulations are found on so-called dendritic spines, thorny protrusions apposed to the presynaptic varicosity. A new study by Ito-Ishida et al. (2012), published in a recent issue of *Neuron*, now provides insights into the *trans*-cellular signaling mechanisms that coordinate these profound rearrangements.

Most previous studies on the cellular dynamics of neuronal synapse formation have focused on filopodial extensions from the dendrite that initiate axo-dendritic contacts and subsequently mature into dendritic spines (Yuste and Bonhoeffer, 2004). By comparison, axonal dynamics during synapse formation are much less defined. The dendrite-centric view has largely emerged from studies on glutamatergic synapses formed onto pyramidal cells in the hippocampus and cortex. However, axo-dendritic dynamics for other synapses differ substantially (Sotelo, 1982; Wierenga et al., 2008). For example, cerebellar Purkinje cells are studded with dendritic spines even in the absence of parallel fibers that constitute their presynaptic partner. Presynaptic varicosities emerge en passant in parallel fiber axons upon contact with the Purkinje cell dendrite. Thus, different synapses adopt different morphogenetic programs. This diversity of morphogenetic programs is mirrored in the diversity of *trans*-synaptic signaling systems that drive synaptogenesis in the brain (Shen and Scheiffele, 2010). Thus, it remains a major question how individual *trans*-synaptic signaling systems instruct specific steps of the synaptic differentiation process.

Ito-Ishida and colleagues (2012) now carefully explore axonal dynamics of parallel fiber synapses in the mouse cerebellum. Previous work implicated a tripartite complex consisting of the presynaptic adhesion molecule neurexin, the extracellular linker Cbln1, and the postsynaptic receptor GluD2 in the formation of parallel fiber synapses. Clustering of axonal neurexins by postsynaptic ligands is sufficient to trigger the assembly of functional presynaptic terminals (Dean et al., 2003). The secreted protein Cbln1 links presynaptic neurexins and postsynaptic GluD2 into the tripartite complex (Uemura et al., 2010). Oligomerization of the components of this complex (GluD2 forms tetramers and Cbln1 hexamers) then drives neurexin clustering and presynaptic differentiation (Lee et al., 2012).

Given that Cbln1 is a secreted factor, it can be exogenously added to slice preparations or the intact brain of Cbln1 knockout mice to acutely trigger the engagement of the neurexin-Cbln1-GluD2 complex (Ito-Ishida et al., 2008). Using live-imaging approaches, Ito-Ishida and colleagues now chronicle morphological