1. Phylogeny  
   IRE2 (gene ERN2, also referred to as Ire1‑β or endoplasmic reticulum–to–nucleus signaling 2) is a member of the IRE1 family of ER membrane–resident proteins that belong to the serine/threonine kinase superfamily. Evolutionarily, the IRE1 family is highly conserved among eukaryotes, with orthologs identified from yeast through to mammals. In mammals, two homologous isoforms exist: IRE1α (ERN1) which is ubiquitously expressed and IRE2 (ERN2), whose expression is primarily restricted to epithelial cells of the gastrointestinal tract (and to a lesser extent in the lung) (siwecka2021thestructureactivation pages 7-9, zhou2021inositolrequiringenzyme pages 1-2). Although IRE2 and IRE1α share comparable domain architectures, the former has evolved distinct tissue‐specific expression patterns and functional capacities. Phylogenetic studies of the eukaryotic kinome reveal that the IRE1 branch – which includes both IRE1α and IRE2 – is part of an ancient set of ER stress sensors, originally present in the Last Eukaryotic Common Ancestor (LECA) (adams2019structureandmolecular pages 2-4). In contrast to many kinases that operate within broadly expressed signaling cascades, IRE2’s restricted expression underscores its specialized role in certain epithelial cell types. This divergence in expression and possibly function marks IRE2 as an evolutionarily distinct member within a conserved kinase family (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 2-4).
2. Reaction Catalyzed  
   The kinase domain of IRE2 catalyzes a phosphorylation reaction that transfers the γ–phosphate from ATP to serine or threonine residues on a substrate protein, following the canonical reaction:  
     ATP + [protein]-(L‑serine or L‑threonine) → ADP + [protein]-(L‑serine/threonine)-phosphate + H⁺  
   This ATP-dependent phosphorylation event is a hallmark of serine/threonine kinases and plays a central role in the regulation of downstream signaling events (adams2019structureandmolecular pages 2-4). In addition to its kinase activity, IRE2 harbors an endoribonuclease domain. Uniquely, rather than cleaving XBP1 mRNA as observed for its paralog IRE1α, IRE2 mediates translational repression by cleaving 28S ribosomal RNA. This dual enzymatic capacity – phosphorylation by the kinase domain and RNA cleavage by the RNase domain – defines the unconventional reaction profile of IRE2 (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 2-4).
3. Cofactor Requirements  
   The catalytic activity of IRE2 is dependent on the binding of ATP, and for efficient phosphoryl transfer the kinase domain requires divalent metal ion cofactors. Magnesium ions (Mg²⁺) serve as essential cofactors, stabilizing the nucleotide substrate within the active site and facilitating the proper orientation required for catalysis. This cofactor dependency is consistent with the general requirements of serine/threonine kinases, where Mg²⁺ acts to coordinate phosphate groups and promote the phosphoryl-transfer reaction (carlesso2018bindinganalysisof pages 1-2).
4. Substrate Specificity  
   IRE2 exhibits dual substrate specificity through its bifunctional catalytic domains. The kinase domain phosphorylates protein substrates on serine/threonine residues; however, a defined consensus substrate motif for this activity has not been delineated in the current literature. In contrast, the endoribonuclease activity of IRE2 is directed towards the cleavage of 28S ribosomal RNA. This RNA cleavage results in translational repression and is central to IRE2’s pro‐apoptotic function. Notably, unlike the closely related IRE1α—which mediates unconventional splicing of X‑box binding protein 1 (XBP1) mRNA—the RNase domain of IRE2 is specialized to target 28S rRNA, thereby distinguishing its substrate specificity from that of its paralog (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 2-4).
5. Structure  
   IRE2 is a type I transmembrane protein that displays a modular organization typical of ER stress sensors. Its overall domain architecture includes an N‑terminal luminal domain that faces the endoplasmic reticulum (ER) lumen and functions as a sensor for protein misfolding, a single transmembrane segment that anchors the protein in the ER membrane, and a cytoplasmic region that houses tandem catalytic domains. The cytoplasmic segment is bifunctional, comprising an N‑terminal protein kinase domain followed immediately by an endoribonuclease (RNase) domain.

The luminal domain is reported to adopt a unique triangular β‑sheet structure that enables it to form stable dimers through hydrogen bonds and hydrophobic interactions. Although detailed high‑resolution structures of full-length IRE2 are not yet available, the full-length structure is presumed to be analogous to that of IRE1α (siwecka2021thestructureactivation pages 7-9). The kinase domain features a bilobal structure consisting of an N‑terminal β‑stranded lobe and a C‑terminal α‑helical lobe. These lobes are separated by a nucleotide‑binding cleft, which is essential for binding ATP and for the catalytic transfer of the phosphate moiety. Within the kinase domain, an activation loop containing well‑conserved motifs – such as the DFG and APE sequences – regulates activity through conformational changes and autophosphorylation events. Residues analogous to Ser724, known to be autophosphorylated in IRE1α, are presumed to play a regulatory role in IRE2 as well (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 6-7).

Following the kinase domain, the RNase domain adopts a predominantly helical conformation. This domain is responsible for RNA substrate recognition and cleavage, and its activity is highly dependent on the formation of active dimeric interfaces that stabilize the catalytic center. Structural studies on IRE1α have demonstrated that RNase activation is accompanied by distinct structural rearrangements, including the formation of a back-to-back dimer. By extrapolation, IRE2 is believed to share this dimerization‑dependent activation mechanism. Overall, the 3D architecture of IRE2 integrates the stress‑sensing capabilities of the luminal domain with the catalytic and regulatory functions of the cytoplasmic kinase and RNase domains, thereby orchestrating both phosphorylation and RNA cleavage in response to ER stress (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 6-7, concha2015longrangeinhibitorinducedconformational pages 9-10, ferri2020activationofthe pages 14-14).

1. Regulation  
   The activity of IRE2 is regulated through a complex interplay of post‑translational modifications, protein–protein interactions, and allosteric mechanisms. Autophosphorylation within the kinase domain is a central regulatory event that triggers conformational changes required for subsequent activation of the RNase domain. This autophosphorylation event promotes dimerization or higher‑order oligomerization of IRE2, which is critical for establishing an active catalytic configuration (adams2019structureandmolecular pages 6-7, siwecka2021thestructureactivation pages 7-9).

Dephosphorylation also plays an important role in modulating IRE2 activity. Specific phosphatases – such as PP2Ce, as identified in studies focused on ER stress phosphatase regulation – dephosphorylate key residues within the kinase activation loop, thereby attenuating the RNase activity and contributing to the fine‑tuning of the stress response (lu2013ppm1lencodesan pages 7-8). Additionally, the luminal domain interacts with ER‑resident chaperones, such as BiP, which bind to and sequester IRE2 in an inactive state under basal conditions; dissociation of BiP in response to unfolded protein accumulation permits IRE2 dimerization and activation (hetz2011theunfoldedprotein pages 8-10).

Moreover, ATP‑competitive ligands have been shown to remodel the kinase front pocket. Such compounds can either stabilize an active (dimer‑promoting) conformation or enforce an inactive state by altering the arrangement of key structural elements such as the C‑helix and activation loop (ferri2020activationofthe pages 14-14, concha2015longrangeinhibitorinducedconformational pages 9-10). These allosteric effects provide an additional layer of regulation, allowing for the selective modulation of the RNase activity through changes in the kinase domain conformation. In summary, the regulation of IRE2 involves cycles of autophosphorylation and dephosphorylation, conformational transitions induced by protein–ligand interactions, and modulation by chaperones – all of which converge to control its dual enzymatic functions (lu2013ppm1lencodesan pages 7-8, hetz2011theunfoldedprotein pages 8-10, ferri2020activationofthe pages 14-14).

1. Function  
   IRE2 (ERN2) plays a uniquely defined role distinct from that of its closely related isoform IRE1α. Rather than participating in the classical unfolded protein response via splicing of X‑box binding protein 1 (XBP1) mRNA, IRE2 is reported to mediate translational repression. It achieves this by using its endoribonuclease domain to cleave 28S ribosomal RNA, an action that results in the downregulation of global protein synthesis. This mechanism of translational repression is coupled to pro‑apoptotic signaling, and it underscores IRE2’s participation in cell fate decisions during ER stress (siwecka2021thestructureactivation pages 7-9, adams2019structureandmolecular pages 2-4).

In addition to its enzymatic roles, IRE2’s tissue‑specific expression further delineates its function. IRE2 is predominantly expressed in epithelial tissues of the gastrointestinal tract, suggesting a specialized role in the regulation of protein synthesis and apoptotic responses in these cells. By effecting 28S rRNA cleavage, IRE2 contributes to the reduction in translation, which may serve as a protective mechanism under conditions of prolonged ER stress that could otherwise lead to cellular damage (zhou2021inositolrequiringenzyme pages 1-2, siwecka2021thestructureactivation pages 7-9). Thus, IRE2 operates as a pro‑apoptotic effector distinct from conventional UPR mediators, channeling ER stress signals toward translational downregulation and programmed cell death.

1. Other Comments  
   Several small‑molecule inhibitors have been developed to target the kinase domain of IRE family members, and ATP‑competitive ligands in particular have been shown to allosterically influence the RNase activity. Notably, research has highlighted that certain inhibitors exhibit marked selectivity between IRE1α and IRE2, with some compounds displaying differences of up to 100‑fold in potency (colombano2019bindingtoan pages 5-6, ferri2020activationofthe pages 12-12). These developments underscore the therapeutic potential of modulating IRE2 activity selectively, especially given its pro‑apoptotic role mediated via 28S rRNA cleavage.  
   Furthermore, the tissue‑restricted expression of IRE2 suggests that its dysregulation may have specific implications in gastrointestinal pathologies or other conditions where aberrant apoptosis could play a role. Although IRE2 has been less extensively characterized than IRE1α in the context of the unfolded protein response, its distinct function – as reported in its protein function annotation – makes it a unique target for further investigation, both in basic research and in the development of clinical interventions.  
   In addition, the interplay between kinase autophosphorylation, dephosphorylation by specific phosphatases (for example, PP2Ce), and allosteric modulation by ligand binding collectively define a regulatory network that may ultimately influence disease outcomes associated with ER stress and apoptotic dysregulation (lu2013ppm1lencodesan pages 7-8, ferri2020activationofthe pages 14-14, concha2015longrangeinhibitorinducedconformational pages 9-10).
2. References
3. siwecka2021thestructureactivation pages 7-9
4. adams2019structureandmolecular pages 2-4
5. adams2019structureandmolecular pages 6-7
6. carlesso2018bindinganalysisof pages 1-2
7. deng2013proteinkinaseand pages 1-1
8. deng2013proteinkinaseand pages 3-3
9. ferri2020activationofthe pages 14-14
10. hetz2011theunfoldedprotein pages 8-10
11. lu2013ppm1lencodesan pages 7-8
12. mendez2015endoplasmicreticulumstressindependent pages 15-21
13. raymundo2020pharmacologicaltargetingof pages 6-8
14. zhou2021inositolrequiringenzyme pages 1-2
15. zhou2021inositolrequiringenzyme pages 2-4
16. zhou2021inositolrequiringenzyme pages 8-9
17. arshad2013rnf13aring pages 1-2
18. benosman2013interleukin1receptorassociatedkinase2 pages 1-3
19. colombano2019bindingtoan pages 5-6
20. concha2015longrangeinhibitorinducedconformational pages 9-10
21. feldman2016structuralandfunctional pages 1-3
22. feldman2016structuralandfunctional pages 11-14

References

1. (siwecka2021thestructureactivation pages 7-9): Natalia Siwecka, Wioletta Rozpędek-Kamińska, Adam Wawrzynkiewicz, Dariusz Pytel, J. Alan Diehl, and Ireneusz Majsterek. The structure, activation and signaling of ire1 and its role in determining cell fate. Biomedicines, 9:156, Feb 2021. URL: https://doi.org/10.3390/biomedicines9020156, doi:10.3390/biomedicines9020156. This article has 147 citations and is from a peer-reviewed journal.
2. (adams2019structureandmolecular pages 2-4): Christopher J. Adams, Megan C. Kopp, Natacha Larburu, Piotr R. Nowak, and Maruf M. U. Ali. Structure and molecular mechanism of er stress signaling by the unfolded protein response signal activator ire1. Frontiers in Molecular Biosciences, Mar 2019. URL: https://doi.org/10.3389/fmolb.2019.00011, doi:10.3389/fmolb.2019.00011. This article has 602 citations and is from a peer-reviewed journal.
3. (adams2019structureandmolecular pages 6-7): Christopher J. Adams, Megan C. Kopp, Natacha Larburu, Piotr R. Nowak, and Maruf M. U. Ali. Structure and molecular mechanism of er stress signaling by the unfolded protein response signal activator ire1. Frontiers in Molecular Biosciences, Mar 2019. URL: https://doi.org/10.3389/fmolb.2019.00011, doi:10.3389/fmolb.2019.00011. This article has 602 citations and is from a peer-reviewed journal.
4. (carlesso2018bindinganalysisof pages 1-2): Antonio Carlesso, Chetan Chintha, Adrienne M. Gorman, Afshin Samali, and Leif A. Eriksson. Binding analysis of the inositol-requiring enzyme 1 kinase domain. ACS Omega, 3:13313-13322, Oct 2018. URL: https://doi.org/10.1021/acsomega.8b01404, doi:10.1021/acsomega.8b01404. This article has 11 citations and is from a peer-reviewed journal.
5. (deng2013proteinkinaseand pages 1-1): Yan Deng, Renu Srivastava, and Stephen H. Howell. Protein kinase and ribonuclease domains of ire1 confer stress tolerance, vegetative growth, and reproductive development in arabidopsis. Proceedings of the National Academy of Sciences, 110:19633-19638, Oct 2013. URL: https://doi.org/10.1073/pnas.1314749110, doi:10.1073/pnas.1314749110. This article has 135 citations.
6. (deng2013proteinkinaseand pages 3-3): Yan Deng, Renu Srivastava, and Stephen H. Howell. Protein kinase and ribonuclease domains of ire1 confer stress tolerance, vegetative growth, and reproductive development in arabidopsis. Proceedings of the National Academy of Sciences, 110:19633-19638, Oct 2013. URL: https://doi.org/10.1073/pnas.1314749110, doi:10.1073/pnas.1314749110. This article has 135 citations.
7. (ferri2020activationofthe pages 14-14): E. Ferri, Adrien Le Thomas, H. Wallweber, E. Day, Benjamin T. Walters, Susan Kaufman, Marie-Gabrielle Braun, K. Clark, M. Beresini, K. Mortara, Y. A. Chen, Breanna Canter, W. Phung, Peter S. Liu, A. Lammens, A. Ashkenazi, J. Rudolph, and Weiru Wang. Activation of the ire1 rnase through remodeling of the kinase front pocket by atp-competitive ligands. Nature Communications, Dec 2020. URL: https://doi.org/10.1038/s41467-020-19974-5, doi:10.1038/s41467-020-19974-5. This article has 35 citations and is from a highest quality peer-reviewed journal.
8. (hetz2011theunfoldedprotein pages 8-10): Claudio Hetz, Fabio Martinon, Diego Rodriguez, and Laurie H. Glimcher. The unfolded protein response: integrating stress signals through the stress sensor ire1α. Physiological Reviews, 91:1219-1243, Oct 2011. URL: https://doi.org/10.1152/physrev.00001.2011, doi:10.1152/physrev.00001.2011. This article has 698 citations and is from a highest quality peer-reviewed journal.
9. (lu2013ppm1lencodesan pages 7-8): Gang Lu, Asuka Ota, Shuxun Ren, Sarah Franklin, Christoph D. Rau, Peipei Ping, Timothy F. Lane, Z. Hong Zhou, Karen Reue, Aldons J. Lusis, Thomas Vondriska, and Yibin Wang. Ppm1l encodes an inositol requiring-protein 1 (ire1) specific phosphatase that regulates the functional outcome of the er stress response. Molecular Metabolism, 2:405-416, Nov 2013. URL: https://doi.org/10.1016/j.molmet.2013.07.005, doi:10.1016/j.molmet.2013.07.005. This article has 48 citations and is from a domain leading peer-reviewed journal.
10. (mendez2015endoplasmicreticulumstressindependent pages 15-21): Aaron S Mendez, Jennifer Alfaro, Marisol A Morales-Soto, Arvin C Dar, Emma McCullagh, Katja Gotthardt, Han Li, Diego Acosta-Alvear, Carmela Sidrauski, Alexei V Korennykh, Sebastian Bernales, Kevan M Shokat, and Peter Walter. Endoplasmic reticulum stress-independent activation of unfolded protein response kinases by a small molecule atp-mimic. eLife, May 2015. URL: https://doi.org/10.7554/elife.05434, doi:10.7554/elife.05434. This article has 68 citations and is from a domain leading peer-reviewed journal.
11. (raymundo2020pharmacologicaltargetingof pages 6-8): Diana Pelizzari Raymundo, Dimitrios Doultsinos, Xavier Guillory, Antonio Carlesso, Leif A. Eriksson, and Eric Chevet. Pharmacological targeting of ire1 in cancer. Trends in Cancer, 6:1018-1030, Dec 2020. URL: https://doi.org/10.1016/j.trecan.2020.07.006, doi:10.1016/j.trecan.2020.07.006. This article has 103 citations and is from a peer-reviewed journal.
12. (zhou2021inositolrequiringenzyme pages 1-2): Zhixin Zhou, Qian Wang, and Marek Michalak. Inositol requiring enzyme (ire), a multiplayer in sensing endoplasmic reticulum stress. Animal Cells and Systems, 25:347-357, Nov 2021. URL: https://doi.org/10.1080/19768354.2021.2020901, doi:10.1080/19768354.2021.2020901. This article has 16 citations and is from a peer-reviewed journal.
13. (zhou2021inositolrequiringenzyme pages 2-4): Zhixin Zhou, Qian Wang, and Marek Michalak. Inositol requiring enzyme (ire), a multiplayer in sensing endoplasmic reticulum stress. Animal Cells and Systems, 25:347-357, Nov 2021. URL: https://doi.org/10.1080/19768354.2021.2020901, doi:10.1080/19768354.2021.2020901. This article has 16 citations and is from a peer-reviewed journal.
14. (zhou2021inositolrequiringenzyme pages 8-9): Zhixin Zhou, Qian Wang, and Marek Michalak. Inositol requiring enzyme (ire), a multiplayer in sensing endoplasmic reticulum stress. Animal Cells and Systems, 25:347-357, Nov 2021. URL: https://doi.org/10.1080/19768354.2021.2020901, doi:10.1080/19768354.2021.2020901. This article has 16 citations and is from a peer-reviewed journal.
15. (concha2015longrangeinhibitorinducedconformational pages 9-10): Nestor O. Concha, Angela Smallwood, William Bonnette, Rachel Totoritis, Guofeng Zhang, Kelly Federowicz, Jingsong Yang, Hongwei Qi, Stephanie Chen, Nino Campobasso, Anthony E. Choudhry, Leanna E. Shuster, Karen A. Evans, Jeff Ralph, Sharon Sweitzer, Dirk A. Heerding, Carolyn A. Buser, Dai-Shi Su, and M. Phillip DeYoung. Long-range inhibitor-induced conformational regulation of human ire1α endoribonuclease activity. Molecular Pharmacology, 88:1011-1023, Dec 2015. URL: https://doi.org/10.1124/mol.115.100917, doi:10.1124/mol.115.100917. This article has 63 citations and is from a domain leading peer-reviewed journal.
16. (ferri2020activationofthe pages 12-12): E. Ferri, Adrien Le Thomas, H. Wallweber, E. Day, Benjamin T. Walters, Susan Kaufman, Marie-Gabrielle Braun, K. Clark, M. Beresini, K. Mortara, Y. A. Chen, Breanna Canter, W. Phung, Peter S. Liu, A. Lammens, A. Ashkenazi, J. Rudolph, and Weiru Wang. Activation of the ire1 rnase through remodeling of the kinase front pocket by atp-competitive ligands. Nature Communications, Dec 2020. URL: https://doi.org/10.1038/s41467-020-19974-5, doi:10.1038/s41467-020-19974-5. This article has 35 citations and is from a highest quality peer-reviewed journal.
17. (arshad2013rnf13aring pages 1-2): M. Arshad, Zhongde Ye, X. Gu, C. Wong, Yang Liu, De Li, Linkang Zhou, Yi Zhang, Wan Ping Bay, V. C. Yu, and Peng Li. Rnf13, a ring finger protein, mediates endoplasmic reticulum stress-induced apoptosis through the inositol-requiring enzyme (ire1α)/c-jun nh2-terminal kinase pathway\*. The Journal of Biological Chemistry, 288:8726-8736, Feb 2013. URL: https://doi.org/10.1074/jbc.m112.368829, doi:10.1074/jbc.m112.368829. This article has 66 citations.
18. (benosman2013interleukin1receptorassociatedkinase2 pages 1-3): Samir Benosman, Palaniyandi Ravanan, Ricardo G. Correa, Ying-Chen Hou, Minjia Yu, Muhammet Fatih Gulen, Xiaoxia Li, James Thomas, Michael Cuddy, Yasuko Matsuzawa, Renata Sano, Paul Diaz, Shu-ichi Matsuzawa, and John C. Reed. Interleukin-1 receptor-associated kinase-2 (irak2) is a critical mediator of endoplasmic reticulum (er) stress signaling. PLoS ONE, 8:e64256, May 2013. URL: https://doi.org/10.1371/journal.pone.0064256, doi:10.1371/journal.pone.0064256. This article has 40 citations and is from a peer-reviewed journal.
19. (colombano2019bindingtoan pages 5-6): Giampiero Colombano, John J. Caldwell, Thomas P. Matthews, Chitra Bhatia, Amar Joshi, Tatiana McHardy, Ngai Yi Mok, Yvette Newbatt, Lisa Pickard, Jade Strover, Somaieh Hedayat, Michael I. Walton, Stephanie M. Myers, Alan M. Jones, Harry Saville, Craig McAndrew, Rosemary Burke, Suzanne A. Eccles, Faith E. Davies, Richard Bayliss, and Ian Collins. Binding to an unusual inactive kinase conformation by highly selective inhibitors of inositol-requiring enzyme 1α kinase-endoribonuclease. Journal of Medicinal Chemistry, 62:2447-2465, Feb 2019. URL: https://doi.org/10.1021/acs.jmedchem.8b01721, doi:10.1021/acs.jmedchem.8b01721. This article has 27 citations and is from a highest quality peer-reviewed journal.
20. (feldman2016structuralandfunctional pages 1-3): Hannah C. Feldman, Michael Tong, Likun Wang, Rosa Meza-Acevedo, Theodore A. Gobillot, Ivan Lebedev, Micah J. Gliedt, Sanjay B. Hari, Arinjay K. Mitra, Bradley J. Backes, Feroz R. Papa, Markus A. Seeliger, and Dustin J. Maly. Structural and functional analysis of the allosteric inhibition of ire1α with atp-competitive ligands. ACS Chemical Biology, 11:2195-2205, Jun 2016. URL: https://doi.org/10.1021/acschembio.5b00940, doi:10.1021/acschembio.5b00940. This article has 101 citations and is from a domain leading peer-reviewed journal.
21. (feldman2016structuralandfunctional pages 11-14): Hannah C. Feldman, Michael Tong, Likun Wang, Rosa Meza-Acevedo, Theodore A. Gobillot, Ivan Lebedev, Micah J. Gliedt, Sanjay B. Hari, Arinjay K. Mitra, Bradley J. Backes, Feroz R. Papa, Markus A. Seeliger, and Dustin J. Maly. Structural and functional analysis of the allosteric inhibition of ire1α with atp-competitive ligands. ACS Chemical Biology, 11:2195-2205, Jun 2016. URL: https://doi.org/10.1021/acschembio.5b00940, doi:10.1021/acschembio.5b00940. This article has 101 citations and is from a domain leading peer-reviewed journal.