## Phylogeny

Mammals possess two IRE1 paralogs, IRE1α (encoded by ERN1) and IRE1β (encoded by ERN2), which likely arose from a gene duplication event and share 39% sequence identity (goupil2024exploringtheire1 pages 2-3, goupil2024exploringtheire1 pages 2-3). Orthologs include IRE1/IRE2 in yeast (*S. cerevisiae*) and the murine Irelpp gene (goupil2024exploringtheire1 pages 7-8, unknownauthors2018thecellularresponse pages 2-4). The kinase and RNase domains are highly conserved across species, while the luminal domain shows significant divergence (goupil2024exploringtheire1 pages 3-5, unknownauthors2018thecellularresponse pages 2-4). According to the kinome classification by Manning et al., ERN2 (IRE2) is classified within the ‘Other’ group of protein kinases (zhou2021inositolrequiringenzyme pages 6-6).

## Reaction Catalyzed

As a serine/threonine kinase, ERN2 catalyzes the phosphotransfer reaction: ATP + [a protein] → ADP + [a phosphoprotein] (zhou2021inositolrequiringenzyme pages 1-2). Its endoribonuclease domain catalyzes the site-specific cleavage of phosphodiester bonds in RNA, such as the cleavage of 28S ribosomal RNA (goupil2024exploringtheire1 pages 2-3, unknownauthors2018thecellularresponse pages 2-4). It is unclear if ERN2 physiologically mediates the splicing of XBP1 mRNA (goupil2024exploringtheire1 pages 2-3).

## Cofactor Requirements

The kinase activity is dependent on ATP for autophosphorylation (prasad2021theendoplasmicreticulum pages 3-3). As a serine/threonine kinase, its catalytic activity requires a divalent cation cofactor such as Mg²⁺ or Mn²⁺ (zhou2021inositolrequiringenzyme pages 1-2, riaz2020roleofendoplasmic pages 3-4).

## Substrate Specificity

The RNase domain of ERN2 cleaves 28S rRNA to inhibit translation (goupil2024exploringtheire1 pages 2-3). The context does not provide information on consensus substrate motifs for the kinase activity of ERN2 based on the priority publication Johnson et al., 2023.

## Structure

ERN2 is a type-I transmembrane protein composed of an N-terminal luminal domain (NLD) for sensing ER stress, a single transmembrane domain (TMD), and a C-terminal cytosolic domain harboring both serine/threonine kinase and endoribonuclease (RNase) activities (prasad2021theendoplasmicreticulum pages 3-3, zhou2021inositolrequiringenzyme pages 2-4). The luminal domain forms a triangular-shaped β-sheet fold that facilitates dimerization (zhou2021inositolrequiringenzyme pages 1-2). The amino acid sequence identity between human IRE1α and IRE1β is 80% for the kinase domain and 61% for the RNase domain (riaz2020roleofendoplasmic pages 1-3). The kinase domain of ERN2 contains nonconserved amino acids compared to IRE1α, which results in impaired catalytic functions (goupil2024exploringtheire1 pages 2-3, goupil2024exploringtheire1 pages 2-3). Specific structural details for ERN2 regarding its activation loop and C-helix are not well-defined (goupil2024exploringtheire1 pages 2-3, goupil2024exploringtheire1 pages 3-5).

## Regulation

Activation of IRE1 proteins requires oligomerization and trans-autophosphorylation (prasad2021theendoplasmicreticulum pages 3-3, goupil2024exploringtheire1 pages 7-8). However, ERN2 displays impaired phosphorylation and oligomerization capabilities compared to IRE1α (grey2020ire1βnegativelyregulates pages 8-11). Specific post-translational modification sites for ERN2 are not well-defined (goupil2024exploringtheire1 pages 2-3). ERN2 acts as a dominant negative modulator of IRE1α by forming heterooligomers with it, which noncompetitively inhibits IRE1α’s RNase activity (goupil2024exploringtheire1 pages 3-5, grey2020ire1βnegativelyregulates pages 8-11). This inhibitory function is structural and does not require ERN2’s own enzymatic activities (grey2020ire1βnegativelyregulates pages 8-11).

## Function

ERN2 is predominantly expressed in the mucosal epithelia of the gastrointestinal and respiratory tracts (goupil2024exploringtheire1 pages 2-3, prasad2021theendoplasmicreticulum pages 3-3). It directly interacts with its paralog, IRE1α (grey2020ire1βnegativelyregulates pages 8-11). Functionally, ERN2 induces translational repression by degrading 28S rRNA (goupil2024exploringtheire1 pages 2-3). By forming heterooligomers with IRE1α, ERN2 attenuates ER stress signaling by acting as a dominant negative regulator (goupil2024exploringtheire1 pages 3-5, grey2020ire1βnegativelyregulates pages 8-11).

## Other Comments

The IRE1 family of proteins is implicated in diseases such as diabetes, cancer, and neurodegeneration, though specific associations for ERN2 are not detailed (riaz2020roleofendoplasmic pages 1-3). Unlike the embryonic lethality observed in IRE1α knockout mice, ERN2 knockout mice are viable (zhou2021inositolrequiringenzyme pages 1-2, zhou2021inositolrequiringenzyme pages 1-2). The impaired catalytic function of ERN2 is attributed to nonconserved amino acids in its kinase domain (goupil2024exploringtheire1 pages 2-3). A kinase-dead mutant of IRE1α exhibits similar behavior to ERN2, with a failure to autophosphorylate and form higher-order oligomers (grey2020ire1βnegativelyregulates pages 8-11).

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