## Phylogeny

– Orthologous genes are documented in human, mouse, rat, bovine, pig, elephant, guinea pig and chicken, indicating deep vertebrate conservation (chakrabarti2011newinsightsinto pages 3-4)  
– The KEN module (pseudo-kinase + RNase) is structurally homologous to Ire1 RNases yet lacks catalytic kinase motifs, placing RNase L in a unique pseudokinase-containing nuclease lineage outside the canonical protein-kinase superfamily (silverman2003implicationsforrnase pages 2-3)  
– Not a protein kinase; no assignment to any group or family within the human kinome (silverman2003implicationsforrnase pages 2-3)

## Reaction Catalyzed

– 2-5A–activated endoribonucleolytic cleavage of single-stranded RNA at UpN sites: ssRNA-UpN + H₂O → 5′-OH RNA fragment + RNA fragment with a 2′,3′-cyclic phosphate; no ATP turnover (cooper2014ribonucleaseland pages 1-2, silverman2003implicationsforrnase pages 2-3)

## Cofactor Requirements

– Activation strictly requires 5′-triphosphorylated 2′,5′-linked oligoadenylates (2-5A) of three or more adenylate units (bisbal2007diversefunctionsof pages 1-2)  
– Catalysis is metal-ion independent (cooper2014ribonucleaseland pages 14-14)  
– Two Walker-A P-loop motifs bind ATP/GTP, but intrinsic kinase activity has not been detected (madsen2008germlinemutationin pages 1-2)

## Substrate Specificity

– Cleavage preference: UU > UA ≫ UG/UC within single-stranded, AU-rich RNA (silverman2003implicationsforrnase pages 2-3)  
– Transcriptome-wide mapping confirms UA/UU consensus independent of extensive secondary structure (cooper2014ribonucleaseland pages 1-2)

## Structure

– Domain organisation: ankyrin repeat domain (aa 1–330), pseudo-kinase lobe (aa 331–535), RNase domain (aa 536–741) (madsen2008germlinemutationin pages 1-2)  
– Nine ankyrin repeats create the high-affinity 2-5A pocket; repeats 2–4 contribute most contacts (ezelle2016therolesof pages 1-3)  
– Catalytic triad His672–Tyr727–Lys789 forms a metal-independent active centre (cooper2014ribonucleaseland pages 14-14)  
– 2-5A bridges ankyrin surfaces of two monomers, enforcing antiparallel dimerisation that unlocks RNase domains (silverman2007ascientificjourney pages 4-5)  
– The ankyrin–pseudo-kinase scaffold functions solely as an allosteric switch, a configuration unique to RNase L (silverman2003implicationsforrnase pages 2-3)

## Regulation

– Femto- to picomolar 2-5A increases dimer affinity by 10⁵–10⁶-fold, converting latent monomer to active nuclease (silverman2003implicationsforrnase pages 2-3)  
– Endogenous inhibitor ABCE1/RLI binds the ankyrin domain and blocks 2-5A-induced dimerisation (ezelle2012pathologiceffectsof pages 1-2)  
– Cellular 2-5A is rapidly degraded by 2′-phosphodiesterases and phosphatases, terminating signalling (ezelle2016therolesof pages 1-3)  
– Proteasome-dependent degradation and stress-induced proteolysis generate 83 kDa and 37 kDa fragments that modulate activity and localisation (liang2006rnaselits pages 5-6, bisbal2007diversefunctionsof pages 14-17)  
– Viral antagonists—vaccinia E3L, influenza A NS1, Theiler’s virus L\* and murine coronavirus ns2—block activation or destroy 2-5A (liang2006rnaselits pages 6-7)

## Function

– Basal expression is low but strongly induced by type I and III interferons in many cell types (ezelle2016therolesof pages 1-3)  
– Upstream: double-stranded RNA activates OAS enzymes to produce 2-5A, which binds and activates RNase L (silverman2003implicationsforrnase pages 1-2)  
– Downstream: RNA fragments engage RIG-I/MDA5–MAVS and the NLRP3 inflammasome, boosting IFN-β and IL-1β production (ezelle2016therolesof pages 1-3)  
– Direct mechanisms: degradation of viral ssRNA, rRNA cleavage-mediated translation arrest, and JNK-dependent mitochondrial apoptosis (silverman2007ascientificjourney pages 4-5)  
– Antibacterial activity enhances clearance of Escherichia coli and Bacillus anthracis via cytokine induction (ezelle2012pathologiceffectsof pages 13-15)  
– Interacts with Filamin A and E3-ligase LNX to support epithelial barrier integrity (ezelle2016therolesof pages 1-3)  
– Binds mitochondrial IF2mt, promoting mitochondrial mRNA turnover and antiproliferative signalling (ezelle2012pathologiceffectsof pages 6-8)  
– Catalytic activity suppresses androgen-receptor transcriptional programmes in prostate cells (ezelle2012pathologiceffectsof pages 6-8)

## Inhibitors

– Endogenous ABCE1/RLI competitively blocks 2-5A binding (ezelle2012pathologiceffectsof pages 1-2)  
– Viral proteins vaccinia E3L, influenza A NS1, Theiler’s virus L\* and coronavirus ns2 antagonise RNase L by preventing activation or degrading 2-5A (liang2006rnaselits pages 6-7)  
– Host 2′-phosphodiesterases limit pathway output by degrading 2-5A activators (ezelle2016therolesof pages 1-3)

## Other Comments

– RNASEL (1q25.3, HPC1) functions as a tumour suppressor; germline mutations M1I, G59S, I97L, E265X, K392R, R462Q, D541E and 471delAAAG are linked to hereditary prostate cancer (carpten2002germlinemutationsin pages 1-1, silverman2003implicationsforrnase pages 3-4)  
– R462Q reduces catalytic activity ~3-fold and is implicated in up to 13 % of prostate-cancer cases (casey2002rnaselarg462glnvariant pages 1-6)  
– Additional associations reported with pancreatic, head-and-neck, breast and cervical cancers (madsen2008germlinemutationin pages 1-2, gusho2020newadvancesin pages 10-10)  
– Chronic fatigue syndrome exhibits elevated RNase L proteolysis and disease-specific 37 kDa fragments (bisbal2007diversefunctionsof pages 14-17)

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