Cell

Structure of the Dual Enzyme Ire1 Reveals the Basis for Catalysis and Regulation in Nonconventional RNA Splicing

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DOI 10.1016/j.cell.2007.10.057

SUMMARY

Ire1 is an ancient transmembrane sensor of ER stress with dual protein kinase and ribonuclease activities. In response to ER stress, Ire1 catalyzes the splicing of target mRNAs in a spliceosome-independent manner. We have determined the crystal structure of the dual catalytic region of Ire1at 2.4 Å resolution, revealing the fusion of a domain, which we term the KEN domain, to the protein kinase domain. Dimerization of the kinase domain composes a large catalytic surface on the KEN domain which carries out ribonuclease function. We further show that signal induced *trans*-autophosphorylation of the kinase domain permits unfettered binding of nucleotide, which in turn promotes dimerization to compose the ribonuclease active site. Comparison of Ire1 to a topologically disparate ribonuclease reveals the convergent evolution of their catalytic mechanism. These findings provide a basis for understanding the mechanism of action of RNaseL and other pseudokinases, which represent 10% of the human kinome.

INTRODUCTION

Endoplasmic reticulum (ER) stress is characterized by the accumulation of toxic misfolded protein aggregates in the ER lumen (Bernales et al., 2006; Ron and Walter, 2007). Ire1 is an evolutionarily conserved, ER stress sensor present in all eukaryotes with both protein kinase and ribonuclease activities (Sidrauski and Walter, 1997). These activities are critical for the induction of the Unfolded Protein Response (UPR), a complex set of homeostatic mechanisms evolved to cope with ER stress in eukaryotes by promoting protein folding and maturation in the ER (Bernales et al., 2006; Ron and Walter, 2007).

Ire1 detects elevated unfolded protein levels in the ER and transmits a signal to the nucleus by splicing mRNAs encoding master transcriptional regulators of the UPR response—Hac1p in yeast and Xbp1 in metazoans—independent of the spliceosome (Calfon et al., 2002; Lee et al., 2002; Sidrauski and Walter, 1997; Yoshida et al., 2001). Ire1 cleaves a single phosphodiester bond in each of two RNA hairpins (with nonspecific base-paired stems and loops of consensus sequence CNCNNGN, where N is any base)(Gonzalez et al., 1999) to remove an intervening intron from target transcripts. A second enzyme, the yeast tRNA ligase Rlg1, joins the adjacent exons to generate a mature transcript (Gonzalez et al., 1999; Sidrauski et al., 1996) (the metazoan homolog of Rlg1 has yet to be identified). Removal of the intron introduces a frame shift that yields a functional activator of the UPR (Calfon et al., 2002; Lee et al., 2002; Mori, 2003; Sidrauski and Walter, 1997; Yoshida et al., 2001). The mature forms of Xbp1 and Hac1 execute the UPR transcription program to upregulate the expression of a multitude of gene products including ER-resident proteins that promote protein folding and maturation and ER-associated proteins involved in protein degradation (Travers et al., 2000).

Ire1 is a type I transmembrane receptor consisting of an N-terminal ER luminal domain, a transmembrane segment and a cytoplasmic region. The cytoplasmic region of Ire1 encompasses a protein kinase domain followed by a C-terminal extension of ~150 residues. The X-ray crystal structure of the luminal domain of yeast and human Ire1 have been determined (Credle et al., 2005; Zhou et al., 2006), shedding light into signal-dependent dimerization/oligomerization of the receptor. No structural information has been forthcoming for the cytoplasmic/enzymatic portion of the protein. The cytoplasmic region of Ire1 contains its catalytic activities and is related to the antiviral ribonuclease RNaseL, which is thought to share a common ribonuclease mechanism. Unlike Ire1, RNaseL lacks protein kinase activity and cleaves single-stranded RNA to inhibit protein synthesis in response to viral stress (Floyd-Smith et al., 1981). While kinase activity seems expendable in the ribonuclease mechanism of RNaseL, and by inference that of Ire1, the structural integrity of the kinase domains of both Ire1 and RNaseL are, paradoxically, essential for their ribonuclease functions. Incidentally, neither Ire1 nor RNaseL display sequence similarity to known nucleases.

Structural and biophysical characterization of the luminal domain of human and yeast Ire1 supports the notion that Ire1 has

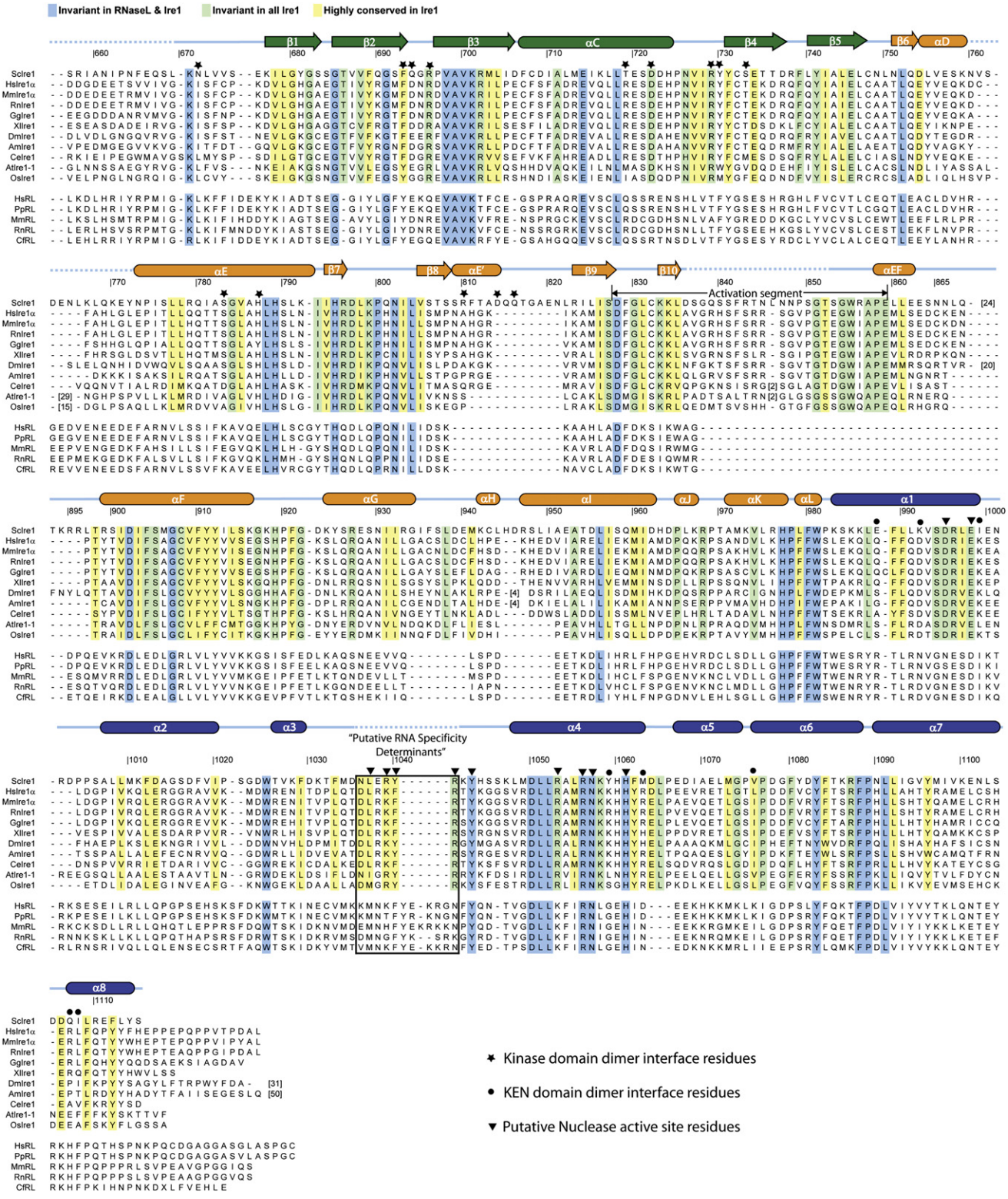

Figure 1. Sequence Conservation in the Dual-Catalytic Core of Ire1

Structure-based sequence alignment of the protein kinase domain and KEN (Kinase Extension Nuclease) domain of Ire1 sequences from *S. cerevisiae* (yeast), *H. sapiens* (human), *M. musculus* (mouse), *R. norvegicus* (rat), *G. gallus* (chicken), *X. laevis* (frog), *D. melanogaster* (fruitfly), *A. mellifera* (honey bee), *C. elegans* (nematode), *A. thaliana* (mouse-ear cress), and *O. sativa* (rice); and RNase L sequences from *H. sapiens* (human), *P. pygmaeus* (orangutan), *M. musculus* (mouse),