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Domain compatibility in Ire1 kinase is critical for the unfolded protein response

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ABSTRACT

The unfolded protein response is a mechanism to cope with endoplasmic reticulum stress. In *Saccharomyces cerevisiae*, Ire1 senses the stress and mediates a signaling cascade to upregulate responsive genes through an unusual *HAC1* mRNA splicing. The splicing requires interconnected activity (kinase and endoribonuclease (RNase)) of Ire1 to cleave *HAC1* mRNA at the non-canonical splice sites before translation into Hac1 transcription factor. Analysis of the truncated kinase domain from Ire1 homologs revealed that this domain is highly conserved. Characterization by domain swapping indicated that a functional ATP/ADP binding domain is minimally required. However the overall domain compatibility is critical for eliciting its full RNase function.
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1. Introduction

The endoplasmic reticulum (ER) is the site for the folding and assembly of secretory and membranous proteins. The environment inside the lumen of the ER is optimized to facilitate folding of the nascent polypeptide into a correct tertiary structure prior to trafficking to the Golgi. Perturbation of the homeostatic environment inside the ER results in accumulation of unfolded proteins that disrupts ER function leading to cell death [1]. An exquisite quality control system ensures that only properly folded proteins exit the ER, and those that are irreparably misfolded are directed to degradation through ER-associated proteasomal degradation and/or autophagy. The cell has evolved the ability to adjust the protein folding capacity to meet fluctuations in the protein folding demand [2]. The unfolded protein response (UPR) is a set of adaptive signaling pathways that evolved to limit accumulation of misfolded proteins by (1) reducing the load on the ER by inhibiting mRNA translation initiation, (2) inducing the transcription of genes encoding protein folding, processing, and trafficking functions, and (3) increasing the degradation rate of irreversibly misfolded proteins. Activation of the UPR is initiated through three ER-localized transmembrane protein sensors Ire1, Perk and ATF6 [3–5]. These proximal effectors of the UPR monitor the protein folding status in the ER through detecting the free pool of the molecular chaperone BiP that is available to facilitate protein folding [6].

Ire1 is the most ancient UPR sensor and is conserved in all eukaryotes. The Ire1 signaling has been well characterized in *Saccharomyces cerevisiae* [6–8]. Its amino terminus resides in lumen ER lumen to sense the protein folding status. The carboxyl terminus resides in the cytosol to initiate a unique signaling through its serine/threonine kinase and endoribonuclease (RNase) activities to induce an unconventional splicing reaction that removes a 252 nucleotide intron from *HAC1* mRNA, encoding a transcription factor that activates UPR-responsive genes [9,10]. The Ire1 kinase comprises 11 subdomains folded into two distinct lobes. A small lobe at the amino terminus is mainly involved in nucleotide binding domain whereas a large lobe at the carboxyl terminus participates in substrate binding [8,11]. Ire1 activation is mediated by oligomerization and trans-autophosphorylation leading to a conformational change that fully elicits the RNase activity [10,12,13].

Here, we describe an inducible Ire1 platform that is amendable for modulating the UPR response in *S. cerevisiae*. Through domain swapping we demonstrate that specific coordination within the kinase domain is critical role for activating the RNase function.

Abbreviations: UPR, unfolded protein response; RNase, endoribonuclease; RT-PCR, reverse transcribed-polymerase chain reaction; *Sca*, *Saccharomyces carlsbergensis*; *Sl*, *Saccharomycodes ludwigii*; *Pt*, *Pachysolen tannophilus*; *Td*, *Torulasopora delbrueckii*; *Wf*, *Wickerhamia fluorescens*

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2. Materials and methods

2.1. Strains and manipulations

S. cerevisiae AWY14 (W303-1A, UPRE-CYC1-LacZ, UPRE-CYC1-LEU 2), *S. cerevisiae* AWY 19 (same as AWY14 except Δire1::kanamycin^r) [14], *Saccharomyces carlsbergensis* (*Sca*), *Saccharomycodes ludwigii* (*Sl*), *Pachysolen tannophilus* (*Pt*), *Torulasopora delbrueckii* (*Td*), *Wickerhamia fluorescens* (*Wf*) were maintained in YEPD. Transformation of recombinant plasmids into the AWY19 strain was performed by the standard lithium acetate method. Strains carrying the plasmid were grown on uracil dropout medium. *Escherichia coli* DH5α was used for propagation and construction of all plasmids.

2.2. Construction of Ire1 expression plasmid

The entire open reading frame of the *IRE1* gene was PCR amplified from *S. cerevisiae* genomic DNA into 3 fragments by Vent DNA polymerase (New England Biolab) using primers shown in Fig. 1 and Table 1. Silent mutations were introduced to the gene to create two unique restriction recognition sites, *Bam*H I *and Xho* I, at positions 2047 and 2421 (relative to the translation initiation site). The fragments were sequentially assembled into pYES-2 plasmid (Invitrogen) downstream of the GAL1 promoter resulting in pYES-*IRE1*. The nucleotide sequence was verified by automated DNA sequencing.

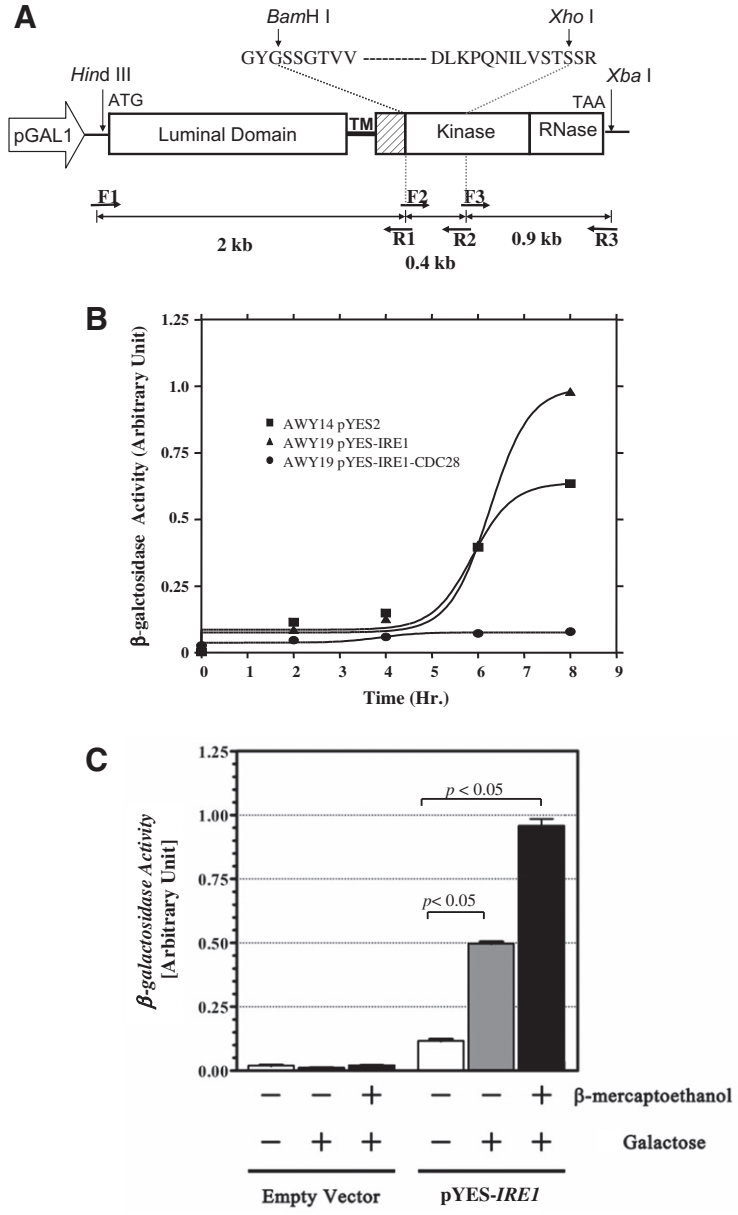

Fig. 1. UPR restoration by inducible Ire1. (A) Construction of the inducible Ire1 expression plasmid. The *S. cerevisiae IRE1* gene was PCR amplified as three consecutive fragments then cloned under GAL 1 promoter in pYES-2 plasmid. *BamH I* and *Xho I* restriction sites (indicate by arrows) were introduced into the kinase subdomains I and VI, respectively. Positions of primers and the approximate size of the amplified fragments are indicated. GYGSSGTVV and DLKPQNILVSTSSR are conserved amino acid sequences in subdomain I and VI used to design degenerate primers. (B) Measure of UPR reporter gene activity. Wild type (*AWY14*) or *ire1* null (*AWY19*) strains with indicated plasmids were cultured in uracil dropout containing 2% raffinose, 1% galactose and 10 mM β-mercaptoethanol. β-Galactosidase activity was measured at indicated time. (C) The UPR activity of the recombinant Ire1 in the presence of absence of ER stress was determined (mean ± S.E.M.) ($n = 3$). ** Represent significant difference of the reporter ($P < 0.05$, $n = 3$) by one-way ANOVA test.

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