SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

Figure S1. Mechanism of *HAC1* mRNA splicing. *HAC1* mRNA is cleaved by Ire1, which leaves a 2', 3'-cyclic phosphate terminus and a hydroxyl terminus at the cleavage sites. Two exons are then joined by yeast tRNA ligase (Rlg1). For this purpose, Rlg1 performs four reactions. The phosphodiesterase activity of Rlg1 hydrolyzes the 2', 3'-cyclic phosphate terminus at the 3'-end of the 5'-exon, yielding 3'-hydroxyl, 2'-phosphatidyl terminus. The polynucleotide kinase activity of Rlg1 promotes the phosphorylation of the hydroxyl terminus at the 5'-end of the 3'-exon with phosphate derived from the γ-position of GTP. Rlg1 then adenylates itself using ATP and transfers its AMP to the phosphorylated 5'-end of the 3'-exon. Following these reactions, Rlg1 ligates two exons by its ligase activity (1, 2, 3, 4). The 2'-phosphate that remains after the reactions by Rlg1 is removed by a 2'-phosphotransferase Tpt1 (5).

Figure S2. Nucleotide requirements for the ligase activity. After cleaving the *XBP1u* RNA with IRE1α, the cleaved product was purified and subjected to *in vitro* splicing reaction with the two Mono Q fractions (fraction 5 and fraction 8). The samples were then subjected to the splicing assay and their relative ligation activity was calculated as described in Material and Methods. The activity observed in the presence of both 1.25 mM ATP and 0.75 mM GTP was taken as 100%. Error bars indicate standard deviations from the average of three independent experiments.

Figure S3. The ligase activity is heat-labile. (**A**) Rabbit erythrocyte lysate (REL) was heated at 96°C for 15 min, cooled on ice and subjected to the splicing assay. Lane 1: assay with untreated REL. Lane 2: assay with heated REL. U and S indicate the positions of the RT-PCR products representing the *XBP1u and XBP1s* RNAs. (**B**) Two Mono Q fractions (fraction 5 and fraction 8) were heated at 96°C for 15 min, cooled on ice and subjected to the splicing assay. The combinations of two Mono Q fractions in the assays shown in this panel are: untreated fraction 5 (lane 3), untreated fraction 8 (lane 4), heated fraction 5 (lane 5), heated fraction 8 (lane 6), untreated fraction 5 and untreated fraction 8 (lane 7), heated fraction 5 and untreated fraction 8 (lane 8), untreated fraction 5 and heated fraction 8 (lane 9) and heated fraction 5 and heated fraction 8 (lane 10).

Figure S4. Schematic representation of *XBP1u* mRNA and the model substrate SL85. (**Left**) Predicted secondary structure of human *XBP1u* mRNA in the region surrounding the IRE1α cleavage sites (6). (**Right**) The GC sequences except the two GC sequences at the IRE1α cleavage sites of the *XBP1u* mRNA fragment were altered in the RNA substrate (SL85), in a manner that should retain the secondary structure of the RNA. The changes made in SL85 were highlighted with red. As far as we examined, these changes did not affect the splicing reaction: SL85 was properly cleaved and re-ligated in our *in vitro* reaction to yield the final spliced product (date not shown). Triangles mark the sites of specific cleavage by IRE1α.

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Figure S1

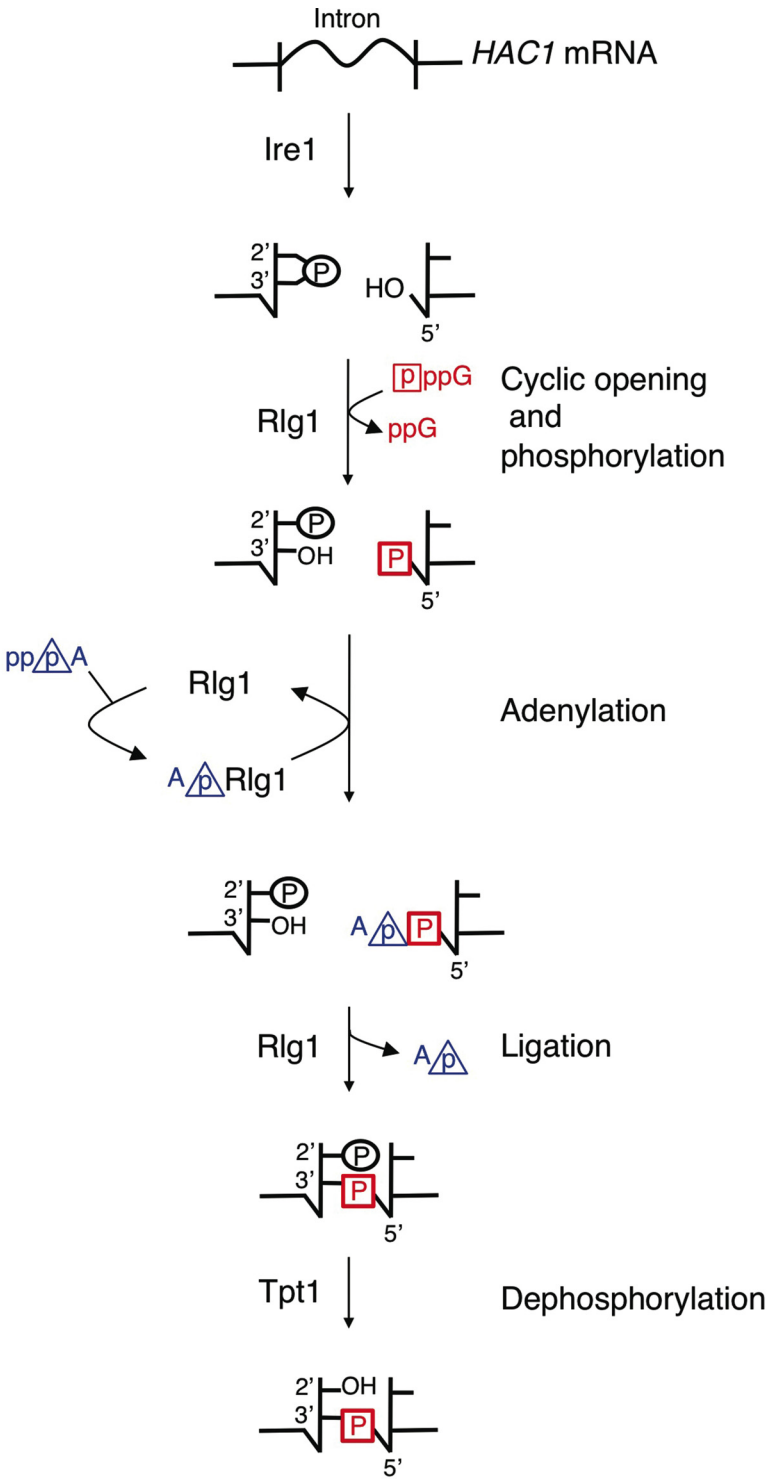
Intron
HAC1 mRNA
Ire1
2'
3'
P
HO
5'
Rlg1
pppG
ppG
Cyclic opening
and
phosphorylation
OH
pp
A
Adenylation
Ligation
Tpt1
Dephosphorylation

Figure S2

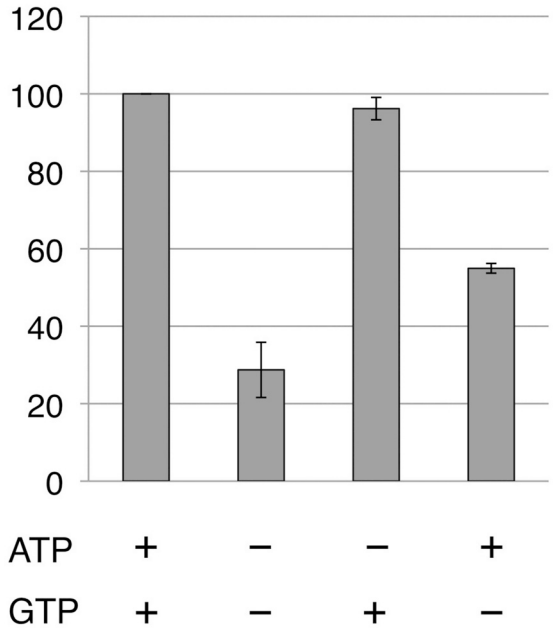

Figure S3

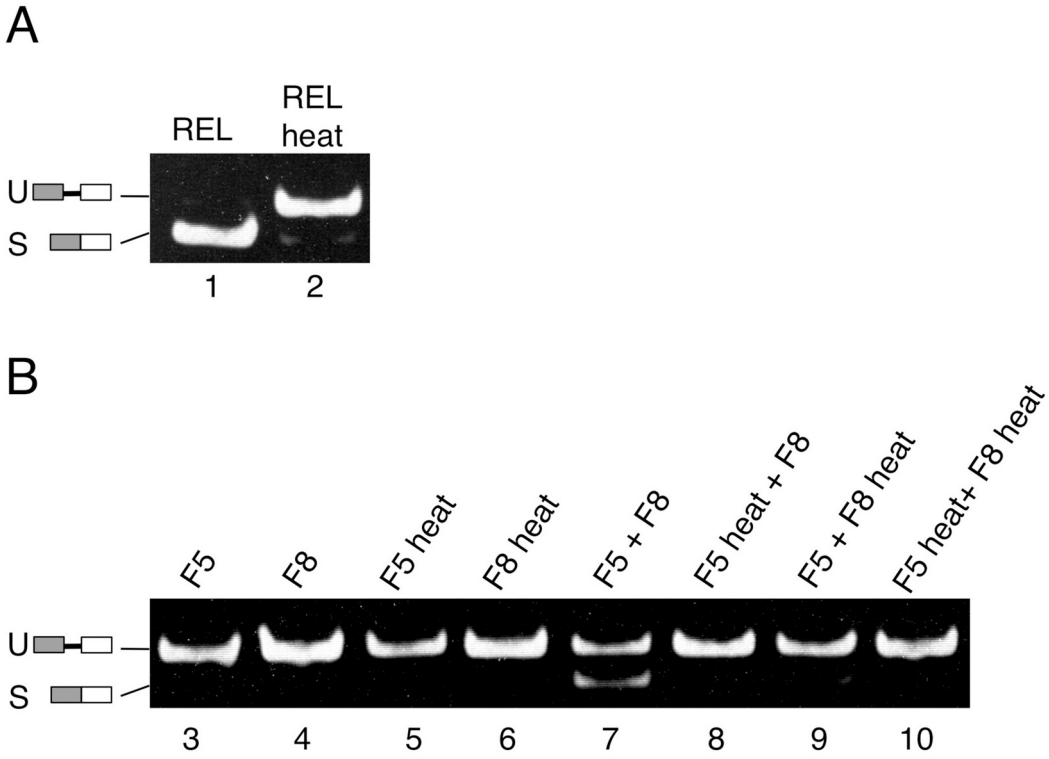
A
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Figure S4