with Bam HI and Sac II and re-ligated after incubation with T4 DNA polymerase to make the control plasmid, pCUP1-GR. To create a 2μ expression plasmid for NMGR, pG1-NMGR, the NMGR DNA fragment was subcloned downstream of the GPD promoter in pG1 (*16*). To create pG1-NMGR526, two primers 5′-ATCAGGATCCAATGTCGGATTC-3′ and 5′-CGGGATCCTCATCCTGCAGTGGCTTGCTGAATC-3′ were used to amplify the $NMGR^{526}$ DNA fragment in a PCR reaction using pG1-NMGR as template. The PCR product was then digested with Bam HI, gel-purified, and subcloned into the Bam HI site of pG1.

18. L. Li and S. Lindquist, data not shown.
19. The Δ*NMSUP35 74D-694* strain (gt12) is a gift from Y. O. Chernoff.
20. M. F. Tuite, C. R. Mundy, B. S. Cox, *Genetics* **98**, 691 (1981).
21. Y. O. Chernoff, S. L. Lindquist, B. Ono, S. G. Inge-Vechtomov, S. W. Liebman, *Science* **268**, 880 (1995).
22. The Hsp104 plasmid was eliminated by repetitively restreaking cells to nonselective media. Plasmid loss was confirmed by testing auxotrophy and by immunoblotting.
23. D. C. Masison, M. L. Maddelein, R. B. Wickner, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 12503 (1997).
24. M. M. Patino and S. Lindquist, unpublished data.
25. J.-J. Liu and S. Lindquist, *Nature* **400**, 573 (1999).
26. D. J. Thiele, *Mol. Cell. Biol.* **8**, 2745 (1988).
27. Each transformant was grown overnight in Synthetic complete media (SC) lacking uracil and leucine (–ura, –leu) at 30°C overnight then diluted into fresh SC (–ura, –leu) to a density of 2×10^6/ml. After 2 hours, deoxycorticosterone (DOC) was added to a final concentration of 10 μM and $CuSO_4$ to the concentrations as indicated in the Fig. 1 legend. Cells were harvested by centrifugation after overnight induction. Extracts were prepared by suspending the cell pellet (from a 3-ml culture) with 200 μl of lysis buffer containing 0.1 M potassium phosphate buffer (pH 7.8), 20% glycerol (v/v), 1 mM dithiothreitol, 2 μg/ml leupeptin, 2 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride. The cell suspension was mixed by vortexing 4 min at 4°C. After centrifugation at 1600*g* for 5 min, 5 μl of the lysate was used to measure β-galactosidase activity, using the Galacto-Light kit from TROPIX (Bedford, MA).
28. Use of a Δ*NMSUP35* strain that already contains the reporter plasmid eliminates a low background of white colonies that arise from problems with two-plasmid cotransformation.
29. B. Gametchu and R. W. Harrison, *Endocrinology* **114**, 274 (1984).
30. We thank K. Yamamoto for BuGR2 antibody; S. Liebman for the N expression plasmid, pEMBL-Δbal2, and the control plasmid, pEMBL-Yex4; M. Patino for the primers of NMGR; Lindquist lab members for comments on the manuscript; and J.-J. Liu for assistance in manuscript preparation. Supported by the Howard Hughes Medical Institute and NIH.

10 August 1999; accepted 7 December 1999

Coupling of Stress in the ER to Activation of JNK Protein Kinases by Transmembrane Protein Kinase IRE1

Fumihiko Urano, XiaoZhong Wang, Anne Bertolotti, Yuhong Zhang, Peter Chung, Heather P. Harding, David Ron*

Malfolded proteins in the endoplasmic reticulum (ER) induce cellular stress and activate c-Jun amino-terminal kinases (JNKs or SAPKs). Mammalian homologs of yeast IRE1, which activate chaperone genes in response to ER stress, also activated JNK, and *IRE1*$\alpha^{-/-}$ fibroblasts were impaired in JNK activation by ER stress. The cytoplasmic part of IRE1 bound TRAF2, an adaptor protein that couples plasma membrane receptors to JNK activation. Dominant-negative TRAF2 inhibited activation of JNK by IRE1. Activation of JNK by endogenous signals initiated in the ER proceeds by a pathway similar to that initiated by cell surface receptors in response to extracellular signals.

cJUN NH_2-terminal kinases [JNKs; also known as stress-activated protein kinases (SAPKs)] constitute a family of signal transduction proteins that are activated under a diverse set of circumstances (*1*). JNKs regulate gene expression through the phosphorylation and activation of transcription factors such as cJUN or ATF2 (*2*) or by regulating mRNA stability (*3*). The physiological significance of JNK signaling has been documented by genetic analysis in *Drosophila* and mice (*4*). Upstream activators of JNK signaling are arranged in a kinase cascade that is similar to that of the yeast pheromone mating pathway (*5*). However, only limited information is available about how proximal signals are coupled to activation of this kinase cascade. The best-characterized link is that between ligation of the tumor necrosis factor (TNF) receptor and activation of JNKs. This link depends on recruitment of adaptor proteins known as TRAFs to the cytosolic side of the ligated receptor (*6*). TRAF2 appears to be specifically important in this regard, because deletion of the gene abolishes JNK activation by TNFα (*7*). The TRAFs activate proximal kinases to initiate a kinase cascade, culminating in JNK phosphorylation and activation (*8*). The mechanistic details of the TRAF-dependent activation of the proximal kinases in the cascade are incompletely understood; however, TRAF effector function depends on the integrity of its NH_2-terminus (*9*).

Stress in the endoplasmic reticulum (ER), induced by perturbations that lead to accumulation of malfolded proteins in that compartment, also activates JNKs (*10*). However, coupling of ER stress to JNK activation is not understood. In yeast, IRE1p, the product of the inositol auxotrophy gene *IRE1*, serves to transduce stress signals from the ER that result in altered gene expression in a pathway known as the "unfolded protein response" (*11, 12*). Two mammalian homologs of yeast IRE1p have been identified: IRE1α (*13*) and IRE1β (*14*). These related transmembrane ER-resident protein kinases are believed to sense ER stress through their conserved lumenal domains. Signal transduction is associated with oligomerization and phosphorylation of the cytosolic portion of IRE1p and increased kinase activity of the protein (*11, 12*). Given their ability to transduce stress signals across the ER membrane and their similarity to classic transmembrane receptors, we examined the possibility that IRE1s also might contribute to JNK activation during ER stress.

Lysates from ER-stressed rat pancreatic acinar AR42J cells treated with thapsigargin (an agent that promotes ER stress by depletion of lumenal calcium stores), tunicamycin (which blocks protein glycosylation), or dithiothreitol (which interferes with disulfide bond formation) all exhibited increased JNK activity (Fig. 1A). Activation of ER stress is revealed by the shift in mobility of the PKR-like ER kinase (PERK), a convenient early marker of ER stress (*15*). Activation of JNKs by ER stress, although always present, varies in magnitude depending on cell type and is particularly pronounced in cells such as AR42J cells, which have a well-developed ER. It is consistently less than that observed in the same cells exposed to ultraviolet (UV) light or the protein synthesis inhibitor anisomycin.

Overexpression of IRE1p or its mammalian homologs leads to their activation independently of ER stress signaling (*13, 14, 16, 17*). Therefore, we overexpressed either form of mammalian IRE1 in cells and measured the kinase activity of a coexpressed exogenous JNK fused to a glutathione *S*-transferase tag (SAPK1β-GST). To limit the analysis of enzyme activity to that present in the transfected cells, the SAPK1β-GST fusion protein was purified by ligand affinity chromatography and then reacted in vitro with the recombinant GST-JUN substrate (*18*). Overexpression of either

Skirball Institute of Biomolecular Medicine, Departments of Medicine, Cell Biology and the Kaplan Cancer Center, New York University Medical School, New York, NY 10016, USA.

*To whom correspondence should be addressed: E-mail: ron@saturn.med.nyu.edu

IRE1β or IRE1α increased SAPK1β-GST activity (Fig. 1B), although levels of activation were less than those imparted by overexpression of a constitutively activated MEKK1, an upstream component of the JNK cascade (*18, 19*). Mutant forms of IRE1 that lack kinase activity and are impaired in induction of the unfolded protein response (*13, 14, 16, 17*) caused little or no activation of SAPK1β even though they were expressed at much higher amounts than were the wild-type proteins (Fig. 1B). Thus activation of the JNKs by IRE1 overexpression appears to depend on some aspect of IRE1 effector function and probably is not a consequence of ER stress caused by overloading the organelle with resident proteins.

To further define the role of IRE1 in JNK activation in response to ER stress, we studied cells deficient in IRE1. The two IRE1 isoforms have different expression patterns; IRE1β expression is limited to the gut, whereas IRE1α has been found in all cells tested (*13*). An allele of *IRE1*α bearing a targeted deletion of the transmembrane domain was created in the germ line of mice by standard embryonic stem (ES) cell techniques and *IRE1*$\alpha^{-/-}$ embryos were produced by heterozygous mating (*20*). Fibroblasts from *IRE1*$\alpha^{-/-}$ embryos had no IRE1α protein detectable by sequential immunoprecipitation and protein immunoblotting with antiserum to the cytosolic portion of the mouse protein (*21*). IRE1α was detectable by this assay in wild-type cells (Fig. 2A). ER stress led to a twofold increase in JNK activity in wild-type fibroblasts, whereas in the *IRE1*$\alpha^{-/-}$ fibroblasts JNK activity was decreased by ER stress (Fig. 2B). The cause for this decrease is not known; however, activation of JNK by UV light and PERK activation by ER stress were indistinguishable in cells of both genotypes (Fig. 2B). This indicates that the mutant cells are capable of responding to stress. We conclude that IRE1α plays an essential role in mediating JNK activation in response to ER stress in embryonic fibroblasts.

To identify possible mediators of the link between IRE1 and JNK activation, we sought to isolate proteins that interact with IRE1. We used the cytosolic effector domain of IRE1β as "bait" in a yeast two-hybrid screen for interacting proteins. We screened a human fibroblast cDNA library and identified 21 interacting clones from a total of 2×10^6 screened (*22*). Two of these encoded different fusions of the full-length human TRAF2 protein to the GAL4 activation domain. Deletion analysis showed that the interaction between the IRE1β COOH-terminus and TRAF2 depended on the TRAF portion of the latter, located at its COOH-terminus (*23*). The so-called TRAF domain, conserved in all TRAFs, mediates their multimerization and interaction with upstream activators (*24*). We coexpressed Flag-tagged TRAF2 with wild-type or mutant forms of IRE1β in mammalian cells and studied their ability to form a stable complex that could be coimmunoprecipitated with antibodies to the epitope-tagged TRAF2. Wild-type IRE1β and a COOH-terminally truncated form that lacks the endonuclease domain required for activation of downstream gene expression (IRE1β$^{\Delta EN}$) were both coimmunoprecipitated with TRAF2 (Fig. 3A). This result is consistent with the observation that IRE1β$^{\Delta EN}$ also activated JNK when overexpressed in cells (*23*). Coexpression of a comparable amount of the Lys^{536} to Ala (K536A) mutant IRE1β that lacks kinase activity with TRAF2 did not result in complex formation (Fig. 3A). This result is consistent with inactivity of the K536A mutant IRE1β as a bait in the two-hybrid screen (*23*) and with inability of the K536A mutant to activate JNK when overexpressed in cells (Fig. 1B). We isolated proteins associated with the endogenous TRAF2 in AR42J cells by immunoprecipitating TRAF2 with an antiserum directed against the NH_2-terminal portion of the protein. Endogenous IRE1α was recovered in the TRAF2 immunoprecipitates only when the cells were treated with an agent that causes ER stress (Fig. 3B). These results suggest that ER stress-induced activation of IRE1 kinase activity is important for TRAF2 recruitment, whereas endonuclease activity of IRE1 is dispensable.

TRAF2 truncated in its NH_2-terminal activation domain functions as a dominant-negative inhibitor of TRAF signaling (*25, 26*). We coexpressed mutant TRAF2, IRE1β, and SAPK1β-GST and measured the latter's activity in cell lysates. In the absence of mutant TRAF2, IRE1β activated SAPK1β-GST (Fig. 4). Increased expression of mutant TRAF2 led to a progressive reduction in SAPK1β-GST activity. Mutant TRAF2 had no effect on the amount of either IRE1β or

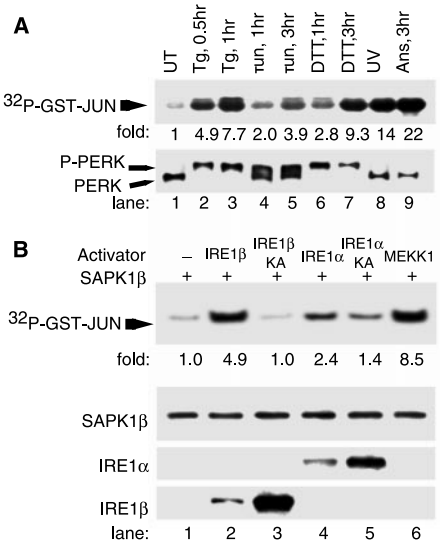

Fig. 1. Activation of JNK by ER stress and IRE1 overexpression. (**A**) (Top) Autoradiogram of endogenous JNK activity in lysates of cells exposed to inducers of ER stress measured by an in vitro kinase assay with purified GST-JUN and [γ-^{32}P]ATP as substrates (*29*). UT, untreated; Tg, thapsigargin (1 μM); Tun, tunicamycin (2.5 μg/ml); DTT, dithiothreitol (10 mM); UV, ultraviolet light (27 J/m^2); Ans, anisomycin (3.8 μM). (Bottom) Protein immunoblot of PERK immunoprecipitated from the same lysates. Activation of PERK by ER stress is reflected in its shift to a lower mobility phosphorylated form, P-PERK. (**B**) (Top) Autoradiogram of JNK activity purified from lysates of 293T cells 40 hours after cotransfection with expression plasmids encoding wild-type or kinase inactive K599A and K536A mutant forms of IRE1α (*13*) and IRE1β (*14*), respectively, and the GST-tagged JNK isoform SAPK1β. JNK (SAPK1β) kinase activity was assayed as described (*18*). Fold activation of SAPK1β was calculated as the ratio of ^{32}P incorporation into the substrate (GST-JUN) between lysates of cells with no IRE1 (lane 1) and the various IRE1 derivatives (lanes 2 to 5). MEKK1 (lane 6) serves as a positive control for JNK activation (*2*). (Bottom) Protein immunoblots of SAPK1β in the kinase reaction and IRE1α and IRE1β derivatives in the input lysates of the kinase reactions. SAPK1β was detected by an antiserum to GST, whereas IRE1α and -β were detected by antibodies to the COOH-terminal portion of the murine proteins. Shown is a typical result of an experiment repeated five times.

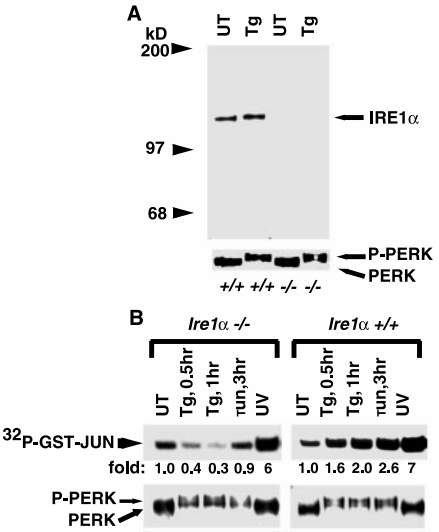

Fig. 2. Requirement of IRE1α for JNK activation in response to ER stress but not UV light. (**A**) IRE1α protein in wild-type and knockout cells. (Top) Immunoprecipitation followed by immunoblot with an IRE1α antiserum from detergent lysates of mouse embryonic fibroblasts with the indicated IRE1α genotypes (*20*) that were untreated (UT) or treated with 1 μM thapsigargin for 1 hour (Tg). (Bottom) Immunoprecipitation and immunoblot of PERK in the same lysates. Migration of molecular size markers is indicated by arrowheads. (**B**) (Top) JNK activity in wild-type and *IRE1*$\alpha^{-/-}$ embryonic fibroblasts exposed to 1 μM thapsigargin (Tg), tunicamycin at 2.5 μg/ml (Tun), or UV light at 27 J/m^2. (Bottom) Immunoblot of PERK immunoprecipitated from the same lysates. Shown are results of a typical experiment repeated five times with cells derived from two independent matched pairs of wild-type and mutant embryos.

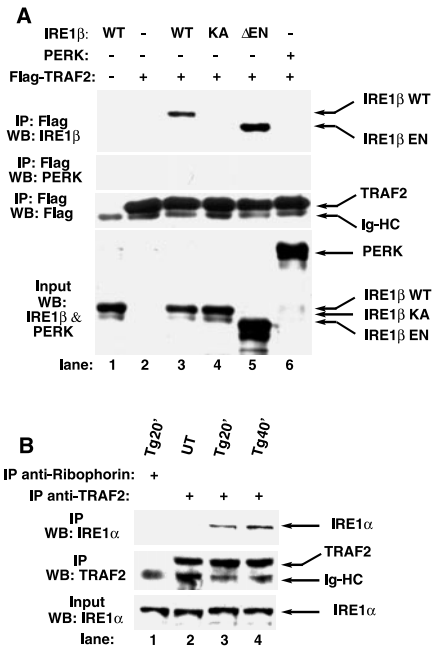

Fig. 3. Interaction of TRAF2 and IRE1 proteins in cells. (**A**) Coimmunoprecipitation of IRE1β and TRAF2 from lysates of 293T cells transfected with expression plasmids encoding the indicated proteins. WT, wild-type IRE1β; KA, K536A mutant IRE1β; ΔEN, mutant IRE1β lacking the endonuclease domain (*14*). PERK (lane 6) served as an indicator for the specificity of the TRAF2-IRE1β interaction. TRAF2, tagged with the Flag epitope, was immunoprecipitated (IP) from cells with the antibody to Flag and the immunoprecipitates were blotted (WB) for the presence of IRE1β or PERK with polyclonal rabbit serum, or TRAF2 using the antibody to Flag (*30*). Proteins on the blot are indicated by the arrows on the right as is the signal from the immunoglobulin heavy chain (Ig-HC). The content of IRE1β and PERK in the lysates is indicated by immunoblotting a sample of the lysate used for the immunoprecipitation reactions (Bottom). (**B**) Endogenous TRAF2 was immunoprecipitated from untreated (UT) and thapsigargin-treated cells (Tg; 1 μM for the indicated time), and the IRE1α present in the immunoprecipitates was revealed by immunoblotting (Top). Immunoprecipitation with anti-Ribophorin serum (lane 1) served as an indicator for specificity of the interaction of IRE1α and TRAF2 in the stressed cells. The amount of TRAF2 recovered in the immunoprecipitate (Middle) and the amount of IRE1α present in a sample of the lysate used in the immunoprecipitation reaction (Bottom) were revealed by immunoblotting.

SAPK1β. These results are consistent with a role for TRAF2, or a related protein acting downstream of IRE1, in mediating the effects of ER stress on JNK activation.

The crystal structure of the COOH-terminal TRAF domain suggests its propensity for multimerization. As such, TRAFs are particularly well suited to interact with targets such as TNF receptors that oligomerize in response to ligand binding (*27*). Clustering of the NH_2-terminal effector domain of TRAF2

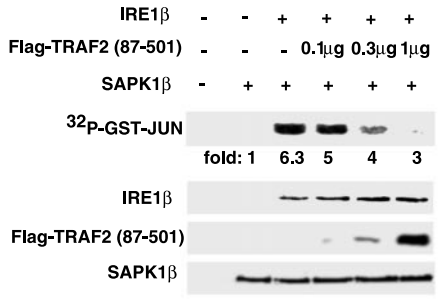

Fig. 4. Inhibition of IRE1-mediated activation of JNK by dominant-negative TRAF2. 293T cells were transfected with expression plasmids for IRE1β, the SAPK1β isoform of JNK, and the indicated amounts of an expression plasmid encoding a Flag-tagged dominant-negative derivative of TRAF2 that lacks the NH_2-terminal effector domain TRAF2 (87–501) (*30*). JNK (SAPK1β) activity was assayed by autoradiography of [^{32}P]GST-JUN phosphorylated in vitro by the SAPK1β purified from the transfected cell lysates (Top). IRE1β and Flag-TRAF2 (87–501) content of the lysate and SAPK1β content of the kinase reaction were measured by immunoblotting (Bottom). Shown is a typical experiment repeated three times.

is sufficient for initiating JNK activation (*28*). Clustering is thought to promote activation of proximal components of the JNK kinase cascade that are bound to the NH_2-terminal effector domain of TRAF2 (*28*). In yeast, ER stress leads to oligomerization of IRE1p (*11*) and a similar event is thought to take place in mammalian cells (*12*). Therefore, stress-induced oligomerization and activation of IRE1 could lead to clustering of TRAF2 that is bound to the COOH-terminal cytoplasmic portion of the IRE1. Thus, activation of JNKs by endogenous ER stress may proceed by a pathway similar to that used by cells to respond to extracellular signals like TNFα. In mouse, IRE1α is an essential gene (*IRE1*$\alpha^{-/-}$ embryos die of unknown cause between days 9.5 and 11.5 of gestation); however, there is no obvious defect in induction of the unfolded protein response in these animals. This leaves open the possibility that in mammalian cells JNK activation in response to ER stress may be an important determinant of cell fate during development.

References and Notes

1. R. J. Davis, *Biochem. Soc. Symp.* **64**, 1 (1999); A. Minden and M. Karin, *Biochim. Biophys. Acta* **1333**, F85 (1997).
2. M. Hibi, A. Lin, T. Smeal, A. Minden, M. Karin, *Genes Dev.* **7**, 2135 (1993); I. Sanchez *et al.*, *Nature* **372**, 794 (1994); S. Gupta, D. Campbell, B. Derijard, R. J. Davis, *Science* **267**, 389 (1995).
3. C. Y. Chen, F. Del Gatto-Konczak, Z. Wu, M. Karin, *Science* **280**, 1945 (1998).
4. D. C. Goberdhan and C. Wilson, *Bioessays* **20**, 1009 (1998); S. Noselli, *Trends Genet.* **14**, 33 (1998).
5. I. Herskowitz, *Cell* **80**, 187 (1995).
6. Z. G. Liu, H. Hsu, D. V. Goeddel, M. Karin, *Cell* **87**, 565 (1996); C. Reinhard, B. Shamoon, V. Shyamala, L. T. Williams, *EMBO J.* **16**, 1080 (1997).
7. S. Y. Lee *et al.*, *Immunity* **7**, 703 (1997); W. C. Yeh *et al.*, *Immunity* **7**, 715 (1997).
8. C. S. Shi and J. H. Kehrl, *J. Biol. Chem.* **272**, 32102 (1997); T. Yuasa, S. Ohno, J. H. Kehrl, J. M. Kyriakis, *J. Biol. Chem.* **273**, 22681 (1998); H. Nishitoh *et al.*, *Mol. Cell* **2**, 389 (1998); H. Liu, Y. C. Su, E. Becker, J. Treisman, E. Y. Skolnik, *Curr. Biol.* **9**, 101 (1999); V. Baud *et al.*, *Genes Dev.* **13**, 1297 (1999).
9. M. Takeuchi, M. Rothe, D. V. Goeddel, *J. Biol. Chem.* **271**, 19935 (1996); H. Y. Song, C. H. Regnier, C. J. Kirschning, D. V. Goeddel, M. Rothe, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9792 (1997).
10. J. M. Kyriakis *et al.*, *Nature* **369**, 156 (1994); R. K. Srivastava *et al.*, *Mol. Cell Biol.* **19**, 5659 (1999).
11. C. E. Shamu and P. Walter, *EMBO J.* **15**, 3028 (1996).
12. R. J. Kaufman, *Genes Dev.* **13**, 1211 (1999).
13. W. Tirasophon, A. A. Welihinda, R. J. Kaufman, *Genes Dev.* **12**, 1812 (1998).
14. X. Z. Wang *et al.*, *EMBO J.* **17**, 5708 (1998).
15. H. Harding, Y. Zhang, D. Ron, *Nature* **397**, 271 (1999).
16. K. Mori, W. Ma, M. J. Gething, J. Sambrook, *Cell* **74**, 743 (1993).
17. J. S. Cox, C. E. Shamu, P. Walter, *Cell* **73**, 1197 (1993).
18. M. Yan *et al.*, *Nature* **372**, 798 (1994).
19. A. Minden *et al.*, *Science* **266**, 1719 (1994).
20. The targeted mutation leads to deletion of the exon encoding the transmembrane domain of murine IRE1α. The 5′ homology arm of the targeting vector is the 6-kb Kpn I–Mun I fragment ending 5′ of the transmembrane domain and the 3′ homology arm is the 4-kb Eco RI–Pst I fragment starting 3′ of the transmembrane domain. Gene targeting was achieved in W4 ES cells and germ line transmission was obtained from two independently derived mutant ES lines with identical phenotypes. Heterozygous mice were intercrossed and embryonic fibroblasts were procured and genotyped at day 9.5 of gestation. Pools of cells, immortalized with simian virus 40 large tumor antigen derived from individual sibling embryos of wild-type and *IRE1*$\alpha^{-/-}$ genotypes were evaluated.
21. Antiserum to IRE1α was raised in rabbits against a bacterially expressed fusion protein consisting of the COOH-terminal 542 residues of mouse IRE1α that encode for the entire kinase-endonuclease domain of the protein fused to a polyhistidine tag.
22. The COOH-terminal IRE1β bait consisted of a fusion between the GAL4 DNA-binding domain and the 460 COOH-terminal residues encompassing the entire kinase and endonuclease domain of IRE1β in the pAS2-1 vector (Clontech). The larger of the two in-frame TRAF2 clones obtained by screening the library made in the pACT2 vector were modified to delete the COOH-terminal region (233 to 501) of TRAF2 (ΔTRAF domain) or to create an in-frame deletion of the first 199 residues (ΔN-term). Interactions were studied in the Matchmaker two-hybrid system 2 (Clontech) according to the manufacturer's instructions.
23. J. S. Oghalai, H.-B. Zhao, T. W. Kutz, W. E. Brownell, data not shown.
24. M. Rothe, S. C. Wong, W. J. Henzel, D. V. Goeddel, *Cell* **78**, 681 (1994).
25. H. Hsu, H. B. Shu, M. G. Pan, D. V. Goeddel, *Cell* **84**, 299 (1996).
26. C. Reinhard, B. Shamoon, V. Shyamala, L. T. Williams, *EMBO J.* **16**, 1080 (1997).
27. Y. C. Park, V. Burkitt, A. R. Villa, L. Tong, H. Wu, *Nature* **398**, 533 (1999).
28. V. Baud *et al.*, *Genes Dev.* **13**, 1297 (1999).
29. M. Hibi, A. Lin, T. Smeal, A. Minden, M. Karin, *Genes Dev.* **7**, 2135 (1993).
30. M. Rothe, V. Sarma, V. M. Dixit, D. V. Goeddel, *Science* **269**, 1424 (1995).

31. Supported by NIH grants ES08681 and DK47119. F.U., H.P.H., and A.B. were supported by fellowships from the Japan Society for the Promotion of Science, NIH, and the European Molecular Biology Organisation, respectively. We thank E. Skolnik and E. Becker (New York University) for advice, R. Kaufman (University of Michigan) for the IRE1α expression plasmids, D. Goeddel (Tularik Corp., San Francisco) for the anti-TRAF2 antiserum, and T. Fujino (Keio University, Tokyo) for the fibroblast cDNA library in pACT2 plasmid.

11 August 1999; accepted 9 December 1999