Supporting Information

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SI Materials and Methods

Phylogenetic Analysis of JIP60. Phylogenetic analysis was performed using the jasmonate-induced protein (JIP)60 sequence using DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) with an e-value cutoff of 1 e^{-19}. The analysis suggested that JIP60 only exists in monocot lineage (Fig. S1). The dicotyledons and Ascomycetes showed similarity only within the ribosome-inactivating protein (RIP)30 domain of JIP60 and are devoid of JIP60. Thus, JIP60 evolved only in the monocot lineage from the type I and II RIPs and adapted properties of both groups. For instance, the RIP30 domain of JIP60 needs to be processed to be functional, as it is found for type I RIPs. Similarly, JIP60 is composed of two domains, a RIP30 domain and a eukaryotic translation initiation factor 4E (eIF4E) domain, and thus resembles type II RIPs, which are composed of a RIP domain and a lectin domain.

Chromosomal Assignment and Comparative Mapping of *JIP60* Genes. *JIP60*-related genes were identified by sequence searches using the barley JIP60 cDNA published by Becker and Apel (1). The two identified genes, *JIP60* and *JIP60-like*, were placed onto the barley consensus map developed by the International Barley Genome Sequencing Consortium (2). Fig. 2 shows results of comparative mapping from left to right the 2012 barley consensus map aligned with the consensus map developed by Rostoks et al. (3). The quantitative trait loci (QTLs) for boron sensitivity and other agronomical traits were placed on the 2005 consensus map based on the trait-mapping information available for the following populations that were used to develop the consensus map: Clipper × Sahara 3771 (4); Morex × Harrington (5); Morex × Steptoe (6); Igri × Danilo (7); Harrington × TR306 (8); and Blenheim × Kym (9). The QTLs for powdery mildew and spot blotch resistance were aligned on the basis of the Cali-sib × Bowman genetic map (10) and the integrated barley genetic map (11), respectively.

Sucrose Density Gradient Centrifugation of Polysomes. Polysomes were isolated from rabbit reticulocyte lysates translating the *RBCS*, *ACTIN*, and *JIP23* model transcripts by Mg^{2+} precipitation as described (12) and loaded onto a discontinuous sucrose step gradient consisting of 2 mL 2 M, 2 mL 1.75 M, 2 mL 1.5 M, 4 mL 1.25 M, 6 mL 1.0 M, 6 mL 0.75 M, and 6 mL 0.5 M sucrose in buffer B (50 mM Hepes·KOH, pH 8.3, 250 mM KCl, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 10 μg/mL cycloheximide, 500 μg/mL heparin). After centrifugation at 60,000 rpm in a Beckman Spinco L 75 centrifuge, rotor Ti 60, for 1 h at 4 °C, the gradient was harvested from bottom to top in a modified Beckman harvesting device with continuous monitoring of the absorbance at 254 nm (2138 Uvicord S; LKB).

Analysis of Polysomal Messengers and Proteins. Protein synthesis was carried out in wheat germ extracts containing 11.2 MBq L-[^{35}S]methionine (37 TBq/mmol; Amersham International). Denaturing agarose gel electrophoresis of RNA was carried out in the presence of formaldehyde (12). After separation, the RNAs were visualized by ethidium bromide staining and blotted onto nitrocellulose membranes (BA-45; Schleicher & Schuell). The membranes in turn were hybridized with an excess of the following ^{32}P-labeled probes: *pHv*611 (1), *pHv*J5 and *pHv*J13 (13), allene oxide synthase (AOS)1/2 and allene oxide cyclase (AOC) (14, 15), *ACTIN* (13), *TUBULIN* (16), and *pHvF08*, a kind gift of L. Hansen, Carlsberg Laboratory, Copenhagen, Denmark. Immunodetection of polysome-bound proteins was done by Western blotting using the antisera indicated in the text.

Coimmunoprecipitation of RNA. Twenty-five grams of leaf segments of 7-d-old plants that had been treated with methyl jasmonate (MeJA) for 96 h and from 56-d-old senescent plants were surface-sterilized in an ice-cold solution of calcium hypochlorite (2.1%; wt/vol) containing sodium chloride (0.1%; wt/vol), washed, and ground under liquid nitrogen until a fine powder was obtained. All of the following operations were performed at 4 °C. After resuspension in buffer containing 50 mM Tris·HCl (pH 8.0), 250 mM KCl, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 10 μg/mL cycloheximide, and 200 μg/mL heparin, the thawed cell homogenate was filtered through two layers of filter gauze (120 μm- and 70 μm-mesh width). The crude cell extract was differentially centrifuged at 2,000, 4,000, and 12,000 rpm for 5 min each in a Sorvall RC-5B centrifuge, using an HB6 rotor, to remove cell debris and organelles. Triton X-100 [1% (vol/vol) final concentration] was added to the final supernatant. After centrifugation at 35,000 rpm in a Spinco L 75B centrifuge, rotor Ti 50, the obtained postribosomal supernatant was subjected to coimmunoprecipitation using antisera against JIP60's bacterially expressed and purified COOH-terminal eIF4E domain or eIF4E from human. eIF4E-bound transcripts were extracted with phenol/chloroform and the messenger portions were purified by oligo-dT cellulose chromatography (17) before being used for Northern hybridization and in vitro translation.

Miscellaneous. One- and two-dimensional SDS/PAGE as well as Western blotting using goat anti-rabbit IgG, rabbit anti-goat IgG, coupled with alkaline phosphatase, or the enhanced chemiluminescence system (ECL; Amersham Biosciences) were carried out as described in ref. 12.

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Fig. S1. Phylogenetic analysis of JIP60 in plant and fungal lineages.

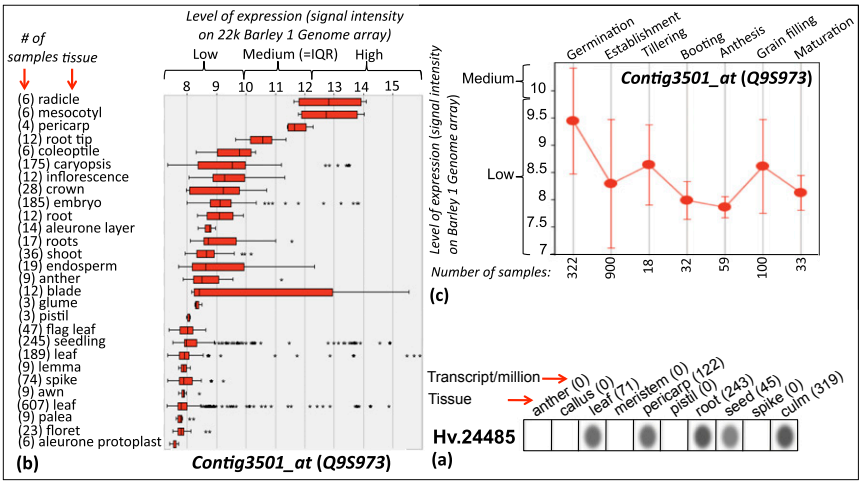

Fig. S2. Virtual expression profile of the barley *JIP60* gene based on the availability of homologous ESTs in the public domain (*A*) and meta-analysis of tissue (*B*) and developmental stage (*C*)-specific transcript data using Genevestigator V3. Transcript analyses shown in *B* and *C* are based on the probe set derived from EST Contig3501_at (new name Barley1_03501) on the Barley1 Genome Array from Affymetrix. Q9S973, UniProt reference; Hv.24485, UniGene reference for barley protein-coding gene LOC548245. Error bars represent standard deviation in the expression data derived from different experiments. IQR, interquartile range.

N-TERMINAL

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  1   maldkvapiv ivtpfnvmtd rydefiekvr kalagtagak vgpkpkskve spvldkgtfp

                                                RNP2-like
 61   veqpprwihv elhgktqgtt tpkpkvairs ddayimgftn stgrwfqlsk tgttyklvdd

                                RNP1-like
121   kavmagfdgn yntlvggvnn lptlnlnkfs maqaaaalwn kastlsggig sdvvddddgd

                                Shiga signature
181   mlrandpvkq avatlavavc eaarfspvsk vvnagwikdk vsvtpdevny ikewgdlsta

                                                              ↓C-TERMINAL
241   llswmdkqyk ddatifkkfn gigitngeea lavvrlvklv irsnmaaapt tdeqilayaq
301   lpkhgrymae vfavripata ggdrpaapsl ctaataaats ftarkkntpr skpaatarvt

                                GTP-EF site
361   wsslghrlat saygpivfnl dlhdgncgqa deeedekntg rivcdaiggd fsnynkaise
421   tvltrcgpae viyavlsngv qgrvdvklag lqsrdevvlv grivarsklf dfgcvlfyne
481   aagvrvrpge lvplarhala vplhmpltie ldirhggsgd eivrgelefk taidglhtgr

      S19 signature
541   lvgvndaefe vtllwseypw
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Fig. S3. Amino acid sequence of barley JIP60 and identification of conserved domains implicated in the catalytic mechanism (Shiga signature), RNA binding [ribonucleoprotein (RNP)1- and 2-like and S19], and GTP fixation (GTP-EF site) (modified after ref. 1). In red are indicated tryptic peptides obtained for the NH_2-terminal 27-kDa band and COOH-terminal 30-kDa band accumulating in vivo (Fig. 3). The arrow marks the presumed NH_2 terminus of JIP60's eIF4E domain.

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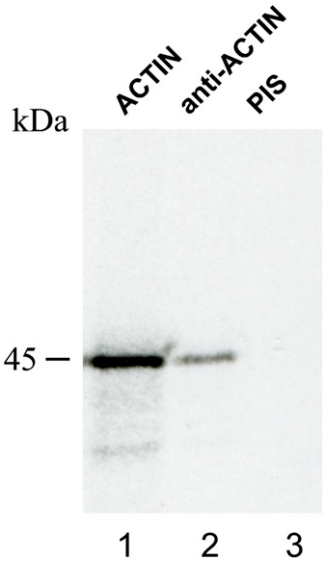

Fig. S4. Characterization of anti-ACTIN serum. Protein was bacterially expressed from the ACTIN cDNA clone described by Shah et al. (1) and used to raise a polyclonal antiserum in rabbits to carry out respective immunoprecipitations. After the immunoreaction, protein was detected by SDS/PAGE and Western blotting (2) using the same anti-ACTIN serum. Lane 1 shows 200 ng of the recombinant protein, lane 2 depicts the result of a respective immunoprecipitation, and lane 3 shows the result of a control precipitation with preimmune serum (PIS).

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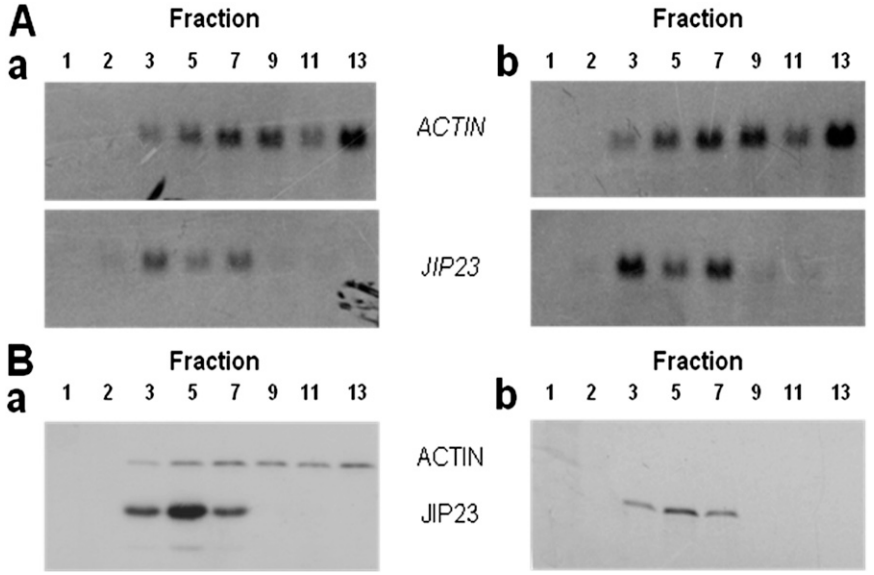

Fig. S5. Polysome binding (*A*) and translation (*B*) of *ACTIN* and *JIP23* transcripts in rabbit reticulocyte lysate incubated without (*a*) or with (*b*) the RIP30 domain of JIP60. Transcripts (*A*) were identified in the indicated polysomal fractions by Northern hybridization using gene-specific probes (1), whereas proteins (*B*) were detected by Western blotting (2) using specific antisera.

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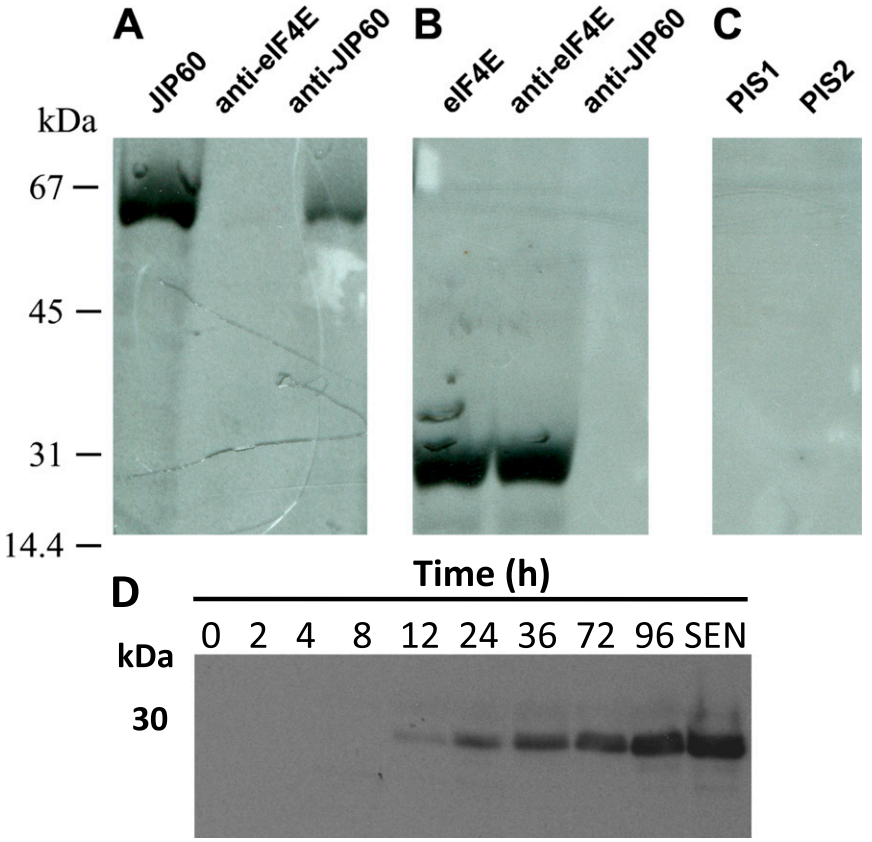

Fig. S6. Cross-reactivity of JIP60 and eIF4E antisera. (*A* and *B*) Two micrograms of the bacterially expressed and purified JIP60 and eIF4E proteins was subjected to immunoprecipitation with the raised JIP60 and eIF4E antisera. (*C*) Control precipitation of JIP60 and eIF4E with respective preimmune sera (PIS1 and PIS2, respectively). Proteins were detected with an enhanced chemiluminescence system (ECL; Amersham Biosciences). (*D*) eIF4E accumulation in leaf extracts from 7-d-old plants that had been treated with MeJA (in h) and from 56-d-old senescent plants (SEN), detected by Western blotting using eIF4E antiserum.

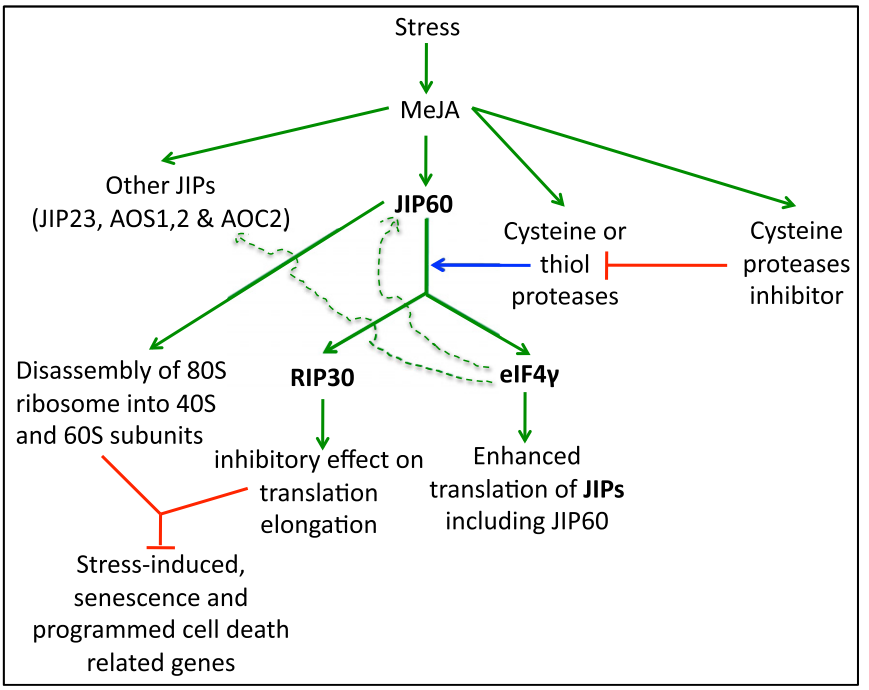

Fig. S7. Diagrammatic representation of the jasmonate-induced signaling cascade showing the sequence of events leading to the translational regulation of *JIP60*, other JIPs, and the downstream genes involved in stress response, senescence, and programmed cell death.

Table S1. P/T values vs. rates of protein synthesis in assay mixtures containing JIP60 and its respective derivatives

Treatment	P/T value*	[^{35}S]Protein, dpm
Control	0.28	35,000
+JIP60	0.03	1,500
+RIP-UP	0.28	34,500
+RIP30	0.24	600
+eIF4E	0.52	54,000

*Rabbit reticulocyte lysates were programmed with *RBCS*, *ACTIN*, and *JIP23* model transcripts, and translations were carried out in the presence of [^{35}S] methionine. Parallel assays contained buffer (control) or JIP60 and respective JIP60, its unprocessed RIP30 domain (RIP-UP), processed RIP30 domain, and eIF4E domain. Polysomes were recovered from the translation mixtures by Mg precipitation and subjected to centrifugation on discontinuous step gradients of sucrose. Then, each gradient fraction was split. One aliquot was used for Northern hybridization using radiolabeled gene-specific probes and determining the amounts of polysome-bound versus unbound/free transcripts. The other aliquot was used for measuring the amount of ^{35}S-labeled protein by liquid scintillation counting. Ribosome:polysome ratios (P/T values) and protein synthesis rates were calculated as described (1).

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