TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h

Mikhail Schepetilnikov, Maria Dimitrova, Eder Mancera-Martínez, Angèle Geldreich, Mario Keller and Lyubov A. Ryabova

Supplementary Materials and Methods

Plant materials and growth conditions

All *Arabidopsis* lines were in Columbia (Col-0) background. DR5:GFP seeds were obtained from the Arabidopsis Biological Resource Center; stock CS9361. The eIF3h T-DNA insertion line *eif3h-1* was described (Kim et al, 2004). The RNA interference (TOR RNAi) line 35-7 was described previously (Deprost et al, 2007; Schepetilnikov et al, 2011). Transgenic lines expressing the auxin response factor 5 (ARF5/ MONOPTEROS) fused to GFP-tag under control of the natural promoter (ARF5::GFP; Schlereth et al, 2010) or GFP under control of the ARF natural promoter/ leader (ARF3:5'-UTR-3xGFP, ARF5:5'-UTR-3xGFP, ARF6:5'-UTR-3xGFP and ARF11:5'-UTR-3xGFP; Rademacher et al, 2011) were kindly provided by Dr. Dolf Weijers from Wageningen Universiteit Laboratory of Biochemistry (the Netherlands). The transgenic line expressing GFP fused to the ER-retention signal under control of the 35S promoter (35S:GFP) was kindly provided by Patrice Dunoyer (IBMP, Strasbourg, France). All plants were grown in the greenhouse under standard conditions.

For immunoprecipitation assays or western blot detection experiments, *Arabidopsis* seedlings were cultured at MS agar for 7 days after germination (7 dag), harvested and ground in liquid nitrogen followed by homogenization in fresh ice-cold extraction buffer [50 mM Tris pH 7.6, 150 mM NaCl, 0.1% NP-40, GM-132 (Sigma), Complete protease inhibitors cocktail (Roche)].

In vitro kinase assay

The *in vitro* kinase assay was carried out at 30°C in buffer (25 mM HEPES pH 7.6, 10 mM $MgCl_2$, 1 mM rATP) with small aliquots of extracts prepared from either wild-type *Arabidopsis* or the *TOR RNAi* silenced 35-7 line with or without 250 nM Torin-1 (Tocris Bioscience) in the presence of 10 µg of recombinant *Arabidopsis* S6K1 produced in *E. coli* as described in Schepetilnikov et al (2011).

Cell culture and time-course experiments

Arabidopsis suspension cells were subcultured weekly at 1/30 dilution in a MS medium at 24°C under constant shacking and light conditions. For phosphorylation kinetic studies, 14 day-old culture was transferred into fresh MS medium with or without induction (20 nM NAA or 250 nM Torin-1). Cells were harvested by centrifugation at 0, 4 and 6 hours after induction and homogenized in the fresh ice-cold Extraction buffer [50 mM Tris pH 7.6, 150 mM NaCl, 0.1% NP-40, GM-132 (Sigma), Complete protease inhibitors cocktail (Roche)] using the Dounce homogenizer. Supernatant was used for the immunoprecipitation assay with anti-eIF3c and anti-TOR antibodies, or for western blot to determine levels and the phosphorylational status of TOR signalling components.

To study ARF::GFP or GFP accumulation *in planta* (Figs 2E and S3D, respectively), 7-dag transgenic ARF seedlings cultured at MS agar plates were transferred into fresh liquid MS media and incubated for 2-3 hours at 24°C under constant light conditions to avoid additional stress. Then seedlings were transferred into fresh liquid MS media with or without 250 nM Torin-1 or 20 nM NAA, and harvested at 0, 4, 6, 8 and 10 hours after induction and grinded in liquid nitrogen followed by homogenization in fresh ice-cold Extraction buffer. Supernatant was used to obtain ARF::GFP protein accumulation kinetics using anti-GFP antibodies and MAPK3/4/6-P, eIF3c, TOR and TOR-P levels using corresponding antibodies by immunodetection analysis.

To measure endogenous mRNA levels by qRT-PCR (Fig. S4B-E), 7 dag *Arabidopsis* Col-0 WT and transgenic *TOR RNAi* seedlings were treated as described above. Total mRNA was isolated from obtained extracts by Trizol (Invitrogen). qRT-PCR was performed according the protocol (see ***Quantitative real-time PCR Analyses***).

The samples for polyribosomal analysis of mRNA and protein redistribution were taken 8 hours after induction and prepared according the protocol (see ***Polyribosome isolation***).

Polyribosome isolation

To prepare polysomes from *eif3h-1* mesophyll protoplasts, the mock-transfected protoplasts and protoplasts co-transformed by plasmids coding for eIF3h, eIF3h-S178D or eIF3h-S178A were incubated during 18 h and lysed in the ice-cold extraction buffer [100 mM Tris-HCl, pH 8.8, 50 mM KCl, 25 mM $MgCl_2$, 5 mM EGTA, 15.4 units/mL heparin, 18 µM cycloheximide, 15.5 µM chloramphenicol, 0.5% NP-40, 1 mM DTT and protease inhibitors cocktail (Roche)] for 15 minutes on ice to obtain cytoplasmic extracts For polyribosome isolation we used 7 dag *Arabidopsis* wild-type Col-0 WT, *TOR RNAi* line 35-7 and *eif3h-1* seedlings not treated

(Mock), or treated with 20 nM NAA (NAA) or 250 nM Torin-1 (Torin-1) or both (NAA/Torin-1) during 8 h. After harvesting, equal amount of fresh material was frozen and ground in liquid nitrogen. For the cytoplasmic extracts, 500 mg of powder were resuspended in the ice-cold extraction buffer. For RNase-treated polysomes, the extraction buffer was supplemented with RNase A (Fermentas). Cell debris was removed by centrifugation. Supernatants were used to control total levels of endogenous mRNA by qRT-PCR and loaded onto 10% to 50% (w/w) sucrose gradients in 100 mM Tris-HCl pH 8.8, 50 mM KCl, 25 mM $MgCl_2$ and centrifuged at 39000 rpm in a Beckman SW41 rotor at 4°C for 3-h. Fractions were collected, precipitated with 10% TCA followed by western blot analysis.

To monitor mRNA loading into polysomes, total RNA isolated from polysomal fractions as indicated were extracted using Trizol (Invitrogen). RNA samples were reverse transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen) with oligo-$(dT)_{18}$ primer (Fermentas). Semi-quantitative RT-PCR was performed with the pair of gene-specific primers to *ARF3* (At2G33860), *ARF5* (At1g19850), *ARF6* (At1G30330), *ARF11* (At2G46530), *bZIP11* (At4G34590), *IAA6* (At1G52830) and *Actin* (At3g18780). Gene-specific primer sequences are available upon request. The PCR conditions are as followed: 2 min, 98°C (first cycle); 30 s, 98°C; 30 s, 56°C; 3 min, 72°C (20 cycles). PCR products were separated on a 1.2% agarose gel and visualized by ethidium bromide staining.

Quantitative real-time PCR analyses

Total RNA was extracted using Trizol (Invitrogen). RNA samples were reverse transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen). cDNA was quantified using a SYBR® Green qPCR kit (EUROGENTEC) and gene specific primers. The level of *ARF*3 (At2G33860), *ARF*5 (At1g19850), *ARF*6 (At1G30330), *ARF*11 (At2G46530), *bZIP*11 (At4G34590), *IAA*6 (At1G52830) mRNAs was monitored by pairs of gene-specific primers. Transcript levels were normalized to that of *ACTIN* (At3g18780) and *GAPDH* (At3g04120). Gene-specific primer sequences are available upon request.

Immunoprecipitation

Plant samples were homogenized in extraction buffer [50 mM Tris pH 7.6, 150 mM NaCl, 0.1% NP-40, GM-132 proteasome inhibitor, Complete protease inhibitors cocktail (Roche)] and insoluble material was removed by centrifugation (30 min, 12000g, 4°C). Lysate was pre-cleaned by incubation with protein A-agarose beads (Roche) at 4°C for 30 min. The supernatant was then incubated with either Normal rabbit serum (RS, Sigma), or anti-wheat

eIF3c, or anti-*At*eIF3h, or anti-mS6K1 or anti-*At*TOR serum prebound to A-agarose beads overnight at 4°C. Immunoprecipitates were washed three times with the extraction buffer, eluted from the beads with 0.1 M Glycine, pH 2.5 and analyzed by western blot.

Western blot assay

Rabbit anti-ARF-GTPase polyclonal antibodies were a kind gift from C. Ritzenthaler (IBMP, Strasbourg, France). Rabbit anti-wheat eIF3c polyclonal antibodies were a kind gift from K. Browning (University of Texas at Austin, TX, USA). Rabbit anti-*At*eIF3h polyclonal antibodies described in Kim et al (2004) were a kind gift from A. von Arnim (University of Tennessee, Knoxville, USA). Rabbit anti-*At*TOR polyclonal antibodies were described previously (Schepetilnikov et al, 2011). Rabbit polyclonal anti-mRaptor and anti-mS6K1 antibodies (see Schepetilnikov et al, 2011) were from Santa-Cruz Biotechnology. Phospho antibodies—anti-mTOR-P-S2448 and anti-mS6K1-P-T389 antibodies—directed against indicated phosphorylated form of either TOR or S6K1 described in Schepetilnikov et al (2011) were from Santa-Cruz Biotechnology. Polyclonal phospho-*At*MPK3/4/6 antibodies (phospho-p44/42 MAPK ERK1/2) used for detection of phosphorylated form of MPK3, MPK4 and MPK6 described in Asai et al (2002) were from Cell Signaling Technology. Polyclonal phospho-(Ser/Thr)Akt/S6K1 substrate (R/KxR/KxxS/T-P) antibody described in Schepetilnikov et al (2011) and used for eIF3h-P detection were obtained from Cell Signaling Technology. Rabbit anti-GFP polyclonal antibodies were provided by D. Gilmer (IBMP, Strasbourg, France).

After SDS–PAGE, gels were transferred to polyvinylidene difluoride membrane PVDF with a Trans-Blot electrophoretic transfer cell (Bio-Rad). Pre-blocking was done with 1% BSA dissolved in 0.1% Tween 20/ PBS, and the membrane was incubated with primary antibodies (1:500 diluted in 1% BSA, 0.1% Tween 20/ PBS). After a series of washes the membrane was incubated with HRP-conjugated secondary anti-rabbit or anti-goat IgG antibodies (Sigma; 1:1000 diluted in 1% BSA, 0.1% Tween 20/ PBS).

Quantification of band density

To quantify the bands on western blots, we applied ImageJ software based analysis (http://rsb.info.gov/ij). The area under curve (AUC) of the specific signal was corrected for the AUC of the loading control (corresponding substrate). The highest value of phosphorylation with the wild type extract was set as 100% and other conditions were recalculated.

The mRNA redistribution in polyribosomal fractions was calculated according to sqRT-PCR band densities quantified by ImageJ and corrected for polysome volume. For each independent gene repetition the highest value of mRNA content in polysomal fraction of NAA-treated sample was set as 100% and other fractions were recalculated.

Two-dimensional gel electrophoresis analysis

Samples for 2D gel experiment were produced from 7 dag *Arabidopsis* seedlings treated for 8 hours with NAA or Torin-1. After harvesting, fresh material was frozen and ground in liquid nitrogen followed by Trizol (Invitrogen) extraction of total protein fraction. For one 2D gel experiment we used 100 µg of total protein resuspended in Iso-Electric-Focusing (IEF) buffer containing 7M Urea, 2M Thiourea, 4% CHAPS, 0.2% TritonX-100. Immobilized 7 cm linear pH gradient (IPG) strips, pH 4-7, were actively rehydrated in IEF buffer at 50V and 20°C for 18 hours. Iso-Electric-Focusing (IEF) was performed for a total of approximately 20 kV-h at 20°C. After IEF, IPG strips were equilibrated in equilibration buffer containing 6 M Urea, 2% SDS, 375 mM Tris pH 8.8, 20% Glycerol, 1% DTT for 15 min at room temperature with a gentle shaking and then 15 min shaking in the same buffer containing 4% iodoacetamide instead of DTT. Equilibrated IPG strips were transferred onto 12.5% uniform polyacrylamide gels and overlaid with 0.5% w/v low-melting-point agarose in running buffer. Gels were run using 10V for 30 minutes then 150V until the dye front run off the bottom of the gels.

Arabidopsis protoplasts and plasmid constructions

Arabidopsis protoplasts from suspension cell cultures (Fig. 7) and mesophyll protoplasts from WT, *TOR RNAi* and *eif3h-1* transgenic plants (Fig. 8) were transfected with plasmid DNA by the PEG method according to Yoo et al (2007). After over-night incubation at 26 °C in WI buffer (4 mM MES pH 5.7, 0.5 M Mannitol, 20 mM KCl) with or without 250 nM Torin-1 or 20 nM NAA transfected protoplasts were harvested by centrifugation and protein extract was prepared in GUS extraction buffer (50 mM NaH_2PO_4 pH 7.0, 10 mM EDTA, 0.1% NP-40). The aliquots were immediately taken for GUS reporter gene assays. GUS activity was measured by a fluorimetric assay described in Pooggin et al (2000). Total RNA from protoplasts was extracted using Trizol. RNA samples were reverse transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen) with oligo-$(dT)_{18}$ primer (Fermentas). sqRT-PCR was performed with the pair of specific primers to the full-length GUS reporter gene. The PCR conditions are as followed: 2 min, 98°C (first cycle); 30 s, 98°C; 30 s, 56°C; 3

min, 72°C (20 cycles). PCR products were separated on a 1.2% agarose gel and visualized by ethidium bromide staining.

The construct pmonoGUS and pmonoGFP were described previously in Schepetilnikov et al (2011). PCR product corresponding to *At*eIF3h was amplified from eIF3h cDNA (At1g10840) with pairs of specific primers and cloned into pmonoGUS to replace GUS and obtain peIF3h construct. peIF3h-S178A and peIF3h-S178D were generated by substitution of Ser at position 178 to Ala (S178A) and to Asp (S178D) respectively in eIF3h ORF by site-directed PCR mutagenesis. PCR product corresponding to *At*S6K1 was amplified from S6K1 cDNA (At3g08730) with pairs of specific primers and cloned into pmonoGUS to replace GUS and obtain pS6K1 construct. pS6K1-T449A and pS6K1-T449E were generated by substitution of Thr at position 449 to Ala (T449A) and to Glu (T449E) respectively in S6K1 ORF by site-directed PCR mutagenesis. pARF5-GUS was cloned by amplifying the *ARF*5/MONOPTEROS leader sequence from *ARF5* cDNA (At1G19850) with pairs of specific primers and inserting it into pmonoGUS upstream of the start codon of the GUS reporter to keep the original context for the ARF5 ORF start-codon. pARF3-GUS was cloned by ampliphying the *ARF*3/ETTIN leader sequence from *ARF3* cDNA (At2G33860) with pairs of specific primers and inserting it into pmonoGUS upstream of the start codon of the GUS reporter to keep the original context for the ARF3 ORF start-codon. The AUG start of either one or both uORFs within the ETTIN leader sequence was replaced by a stop codon using site-directed PCR mutagenesis to generate respectively pARF3ΔAUG1-GUS, pARF3ΔAUG2-GUS and pARF3Δ(AUG1+2)-GUS constructs. pMAGDIS-GUS was generated by inserting the leader sequence GACACGCTGAAATCACCAGTCTCTCTC TACAAATCTATCTCTCTCTATTTTCTCGAGAATA**ATGGCCGGCCGCATTAGCTA G**TTCCCAGATAAGGGAATTAGGGTTCTTATAGGGTTTCGCTAGC with MAGDIS uORF at the position 61 into pmonoGUS upstream of the start codon of the GUS reporter. pMAGRIS-GUS was generated by substitution of Asp to Arg in MAGDIS uORF by site-directed PCR mutagenesis.

Assay for root gravitropism

Root length was assayed for 14 dag seedlings of wild type and 35-7 *TOR RNAi* and *eif3h-1* lines (Fig. 9A). Root tip reorientation experiments were performed on 7 dag seedlings (Fig. 9B). Seeds were sterilized, vernalized in the dark at 4°C for 48-h and then germinated vertically on MS agar plates at 22°C for 4 days. Vertically grown seedlings were transferred to the new plates containing MS agar with of without 250 nM Torin-1 (Tocris Bioscience).

The plates were then placed in the dark at 22°C maintaining the same root orientation and allowed to grow vertically for a further 2 days. The plates were then turned through 90° and grown for a further 24 hours under the same growth conditions before scoring for reorientation of root growth.

To observe GFP signal during gravitropism (Fig. 9C), 4 dag vertically grown seedlings of DR5:GFP and 35S:GFP lines were mounted in 0.8% agarose in liquid MS medium on a slide with or without 250 nM Torin-1, kept vertically for a further 2 days in the dark following rotation 90° and imaged 4, 6 and 12 hours post reorientation using LSM700 confocal microscope (Zeiss, Germany).

Molecular modelling

The 3D structure of *Arabidopsis* eIF3h was created using Modeller (Sali et al, 1995) and graphical representation was drawn using PyMOL (http://www.pymol.org). Support structure for modelling the central region (1-200 aa) of eIF3h was found with NIH BLAST (the 3D-structure of the MPN domain of human Mov34 was used as a template).

Yeast two-hybrid assay

To generate pGAD-eIF3h, PCR product corresponding to eIF3h was ampliphied from *At*eIF3h cDNA (At1g10840) with pairs of specific primers and cloned into the pGADT7 (Clontech) as in-frame fusion with the AD-domain. PCR products corresponding to NTOR, CTOR, S6K1 and Raptor1B were amplified from *At*TOR cDNA (At1G50030), *At*S6K1 cDNA (At3G08730) and *At*Raptor1B cDNA (At3g08850) respectively with pairs of specific primers and cloned into the pGBKT7 (Clontech) as in-frame fusion with the BD-domain to obtain pGBK-NTOR, pGBK-CTOR, pGBK-S6K1 and pGBK-Raptor1B.

Yeast two-hybrid protein interaction assays were performed according to Park et al (2001). Constructs containing eIF3h fused to the GAL4 AD-domain and NTOR, CTOR, S6K1 and Raptor1B fused to the BD-domain were co-transformed into AH109 cells. Transformants were selected onto SD-Leu-Trp plates. Surviving yeast colonies were picked as primary positives and transferred on SD-Leu-Trp-Ade selection plates to score protein interaction.

Supplemental references

Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolai M, Bedu M, Robaglia C, and Meyer C. (2007) The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Rep* 8: 864–870

Kim TH, Kim BH, Yahalom A, Chamovitz DA, and von Arnim AG. (2004) Translational regulation via 5' mRNA leader sequences revealed by mutational analysis of the Arabidopsis translation initiation factor subunit eIF3h. *Plant Cell* 16: 3341–3356

Park HS, Himmelbach A, Browning KS, Hohn T, Ryabova LA (2001) A plant viral "reinitiation" factor interacts with the host translational machinery. *Cell* **106**: 723–733

Pooggin MM, Hohn T, and Futterer J. (2000) Role of a short open reading frame in ribosome shunt on the cauliflower mosaic virus RNA leader. *J. Biol. Chem.* 275: 17288–17296

Rademacher E, Möller B, Lokerse AS, Llavata-Peris CI, van den Berg W, and Weijers D. (2011) A cellular expression map of the Arabidopsis AUXIN RESPONSE FACTOR gene family. *Plant J.* 68: 597-606

Sali A, Potterton L, Yuan F, van Vlijmen H, and Karplus M. (1995) Evaluation of comparative protein modeling by MODELLER. *Proteins* 23: 318–326

Schepetilnikov M, Kobayashi K, Geldreich A, Caranta C, Robaglia C, Keller M, and Ryabova LA. (2011) Viral factor TAV recruits TOR/S6K1 signalling to activate reinitiation after long ORF translation. *EMBO J.* 30: 1343–1356

Schlereth A, Möller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jürgens G, and Weijers, D. (2010). MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. Nature 464: 913–916

Yoo SD, Cho YH, and Sheen J. (2007) Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat Protoc* 2: 1565–1572

Supplementary Figures and legends

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Figure S1

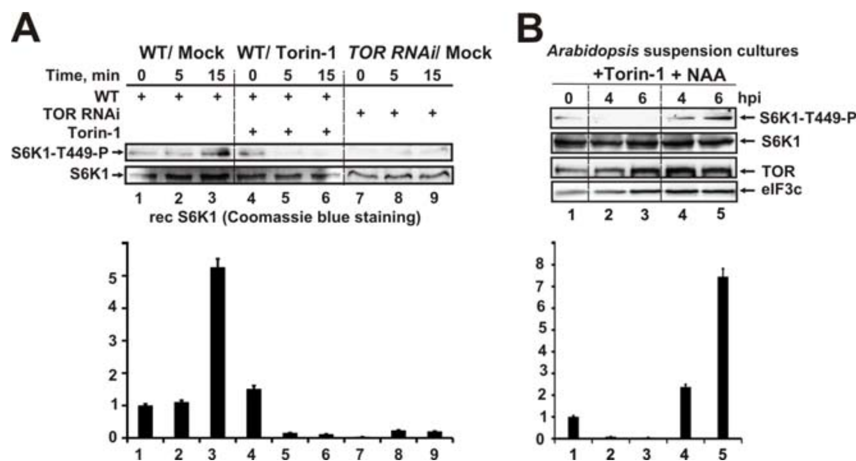
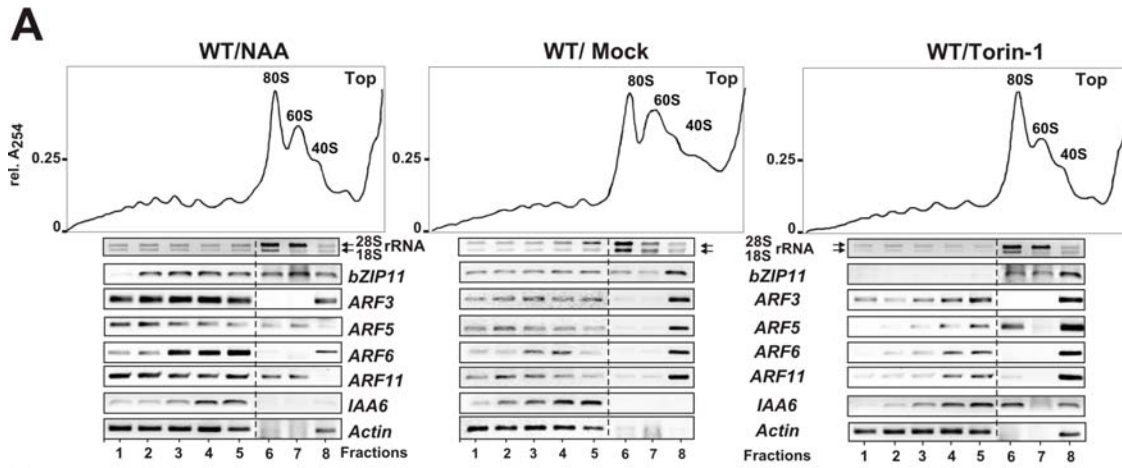
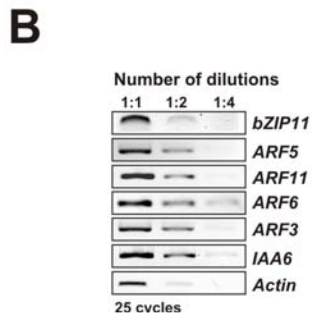


Figure S2





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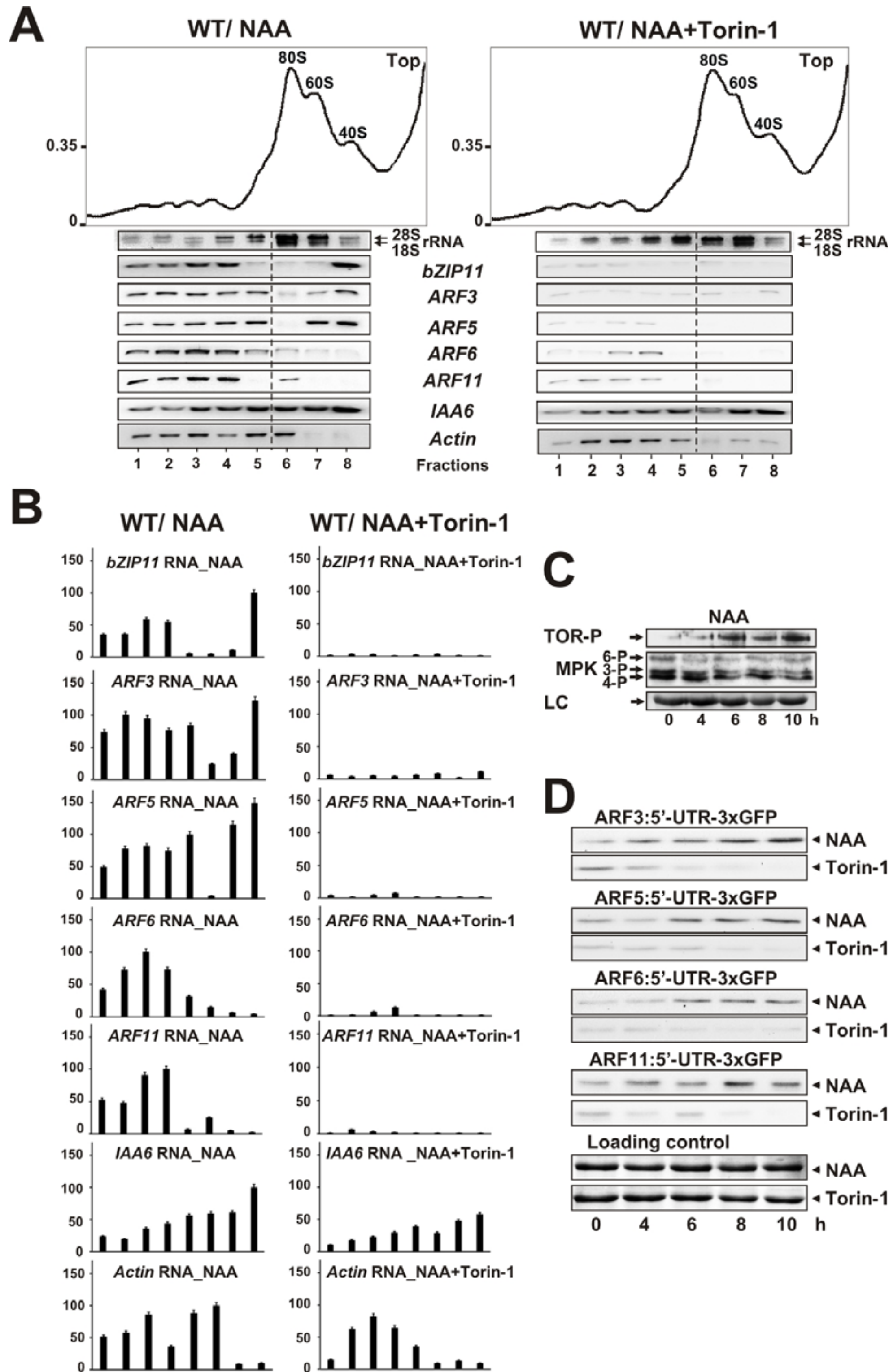


Figure S4

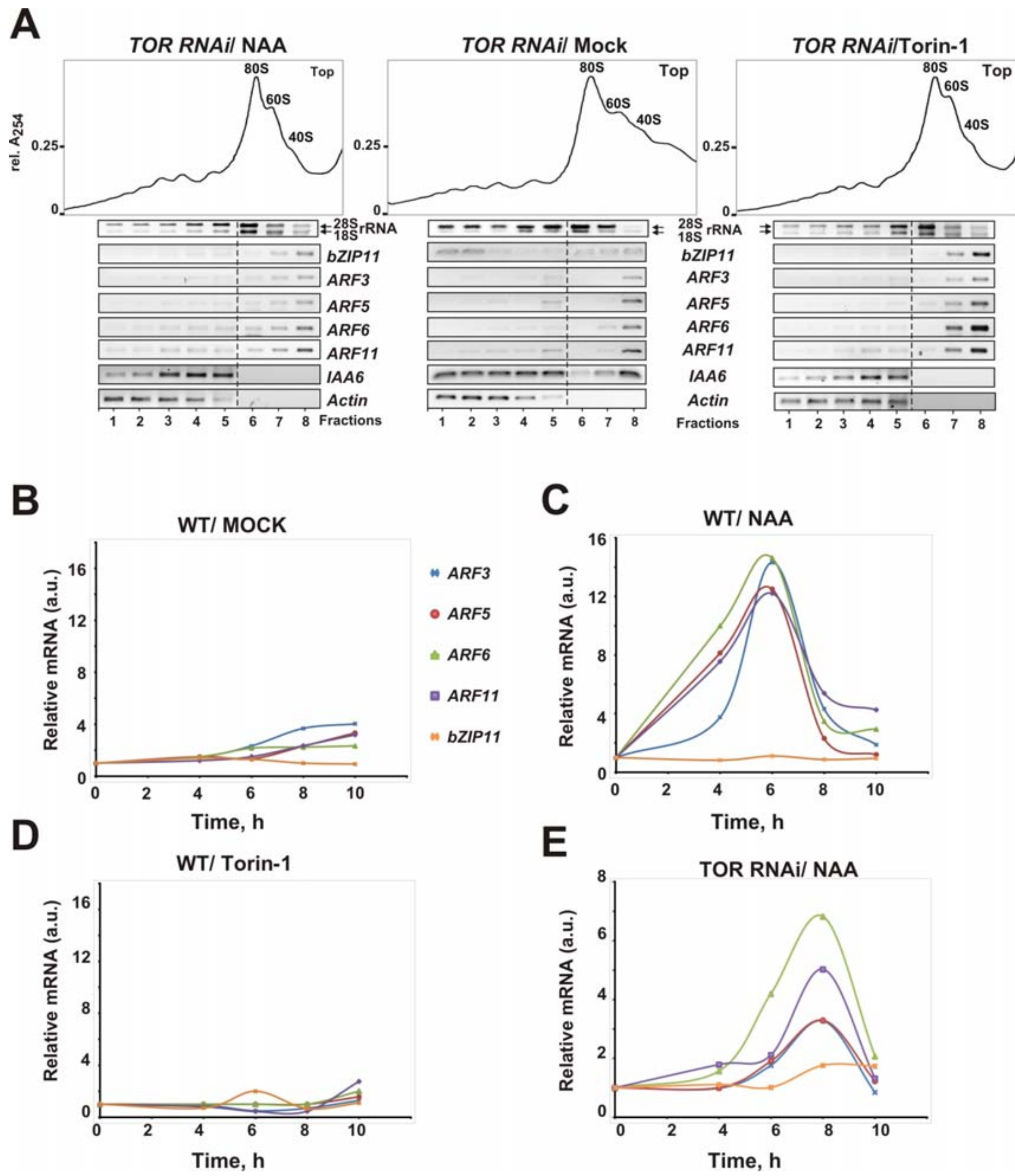
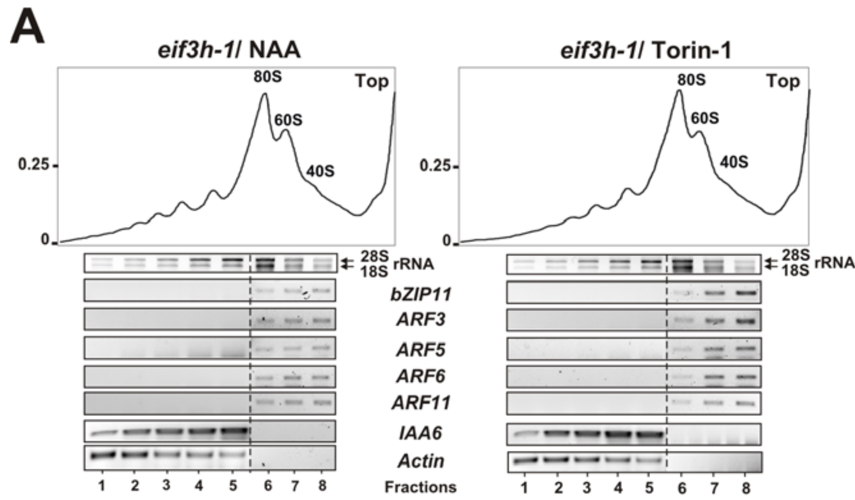
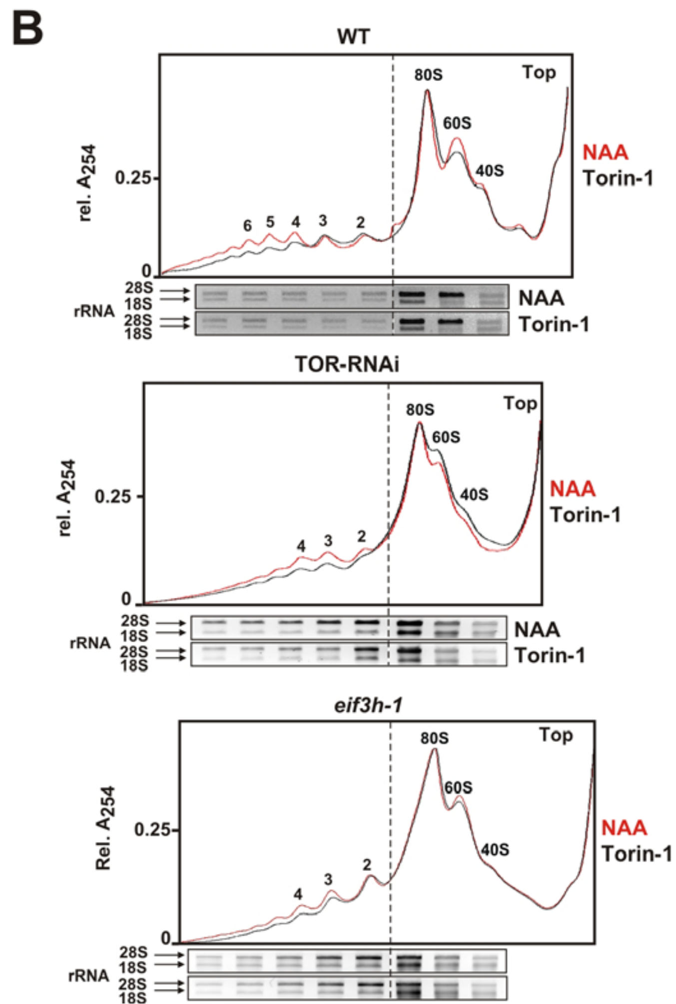


Figure S5





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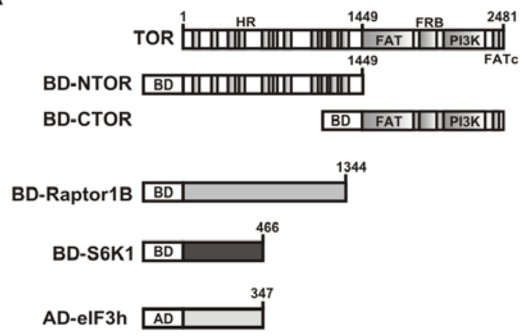


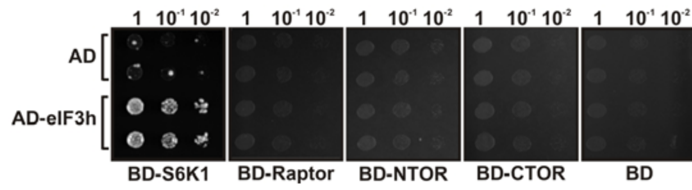
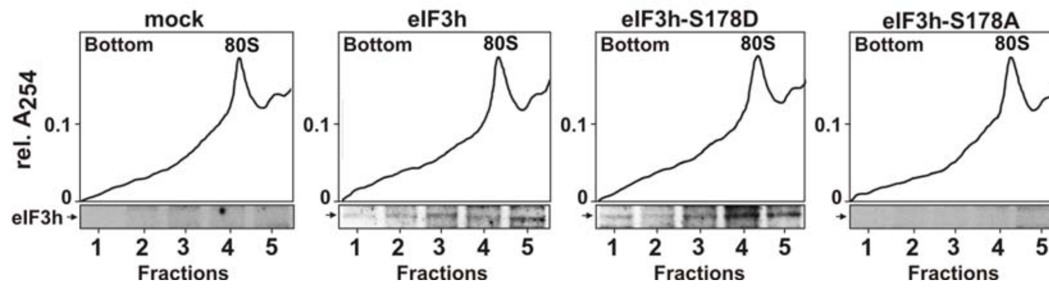


Figure S7



Supplementary Figure legends

Figure S1 (related to Figure 1) (**A**) Phosphorylation of recombinant S6K1 was assayed in *Arabidopsis* extracts of WT (± Torin-1) or *TOR RNAi* seedlings during 0, 5, 15 minutes. *Upper panel* Immunoblot analysis of recombinant S6K1 phosphorylation at TOR-specific T449. S6K1 and S6K1-T449-P were visualized by Coomassie blue staining and anti (R/KxR/KxxT/S-P) antibodies, respectively. *Lower panel* Quantification of western blot results. The amount of specific signal for phosphorylated S6K1 was corrected for sample loading in the displayed diagrams. The data shown are the means of three independent blots: error bars indicate sd. The value for S6K1-T449-P in *Arabidopsis* extract (no incubation) was set as 100%. The respective band densitometry analysis performed with Image J software. (**B**) Phosphorylation of S6K1 at T449 in *Arabidopsis* suspension cultures is up-regulated by auxin in Torin-1-sensitive manner. *Upper panel* Western blot analysis of S6K1-T449-P phosphorylation levels, as well as S6K1, TOR and eIF3c levels at 0, 4 and 6 h post induction (hpi) with Torin-1 or NAA. *Lower panel* Quantification of western blot results presented in *Upper panel* using the band densitometry analysis performed with Image J software as in (**A**). The value for S6K1 phosphorylation in *Arabidopsis* suspension culture (no incubation) was set as 100%. The amount of specific signal for phosphorylated S6K1 was corrected for sample loading in the displayed diagrams. The data shown are the means of three independent blots: error bars indicate sd.

Figure S2 (related to Figure 2) *ARF3*, *ARF5*, *ARF6*, *ARF11*, *bZIP11* mRNA accumulation in polysomes is stimulated by auxin in Torin-1-sensitive manner. (**A**) Distribution of mRNAs—*ARF3*, *ARF5*, *ARF6, ARF11*, *bZIP11*, *IAA6* and *Actin* analyzed in polysome gradient fractions from extracts prepared from 7 dag seedlings treated (or not, Mock) with either auxin (NAA) or Torin-1 for 8 h was analyzed by semiquantitative reverse transcription polymerase chain reactions (sqRT-PCR; quantifications see in Fig. 2B) in each gradient fraction. mRNAs were visualized by ethidium bromide (EtBr). sqRT-PCR was done in three replicates. The ribosomal profiles, distribution of 18S and 28S rRNAs within ribosomal profiles are shown. (**B**) sqRT-PCR analyses of different total mRNAs in extracts prepared from WT seedlings at different saturation conditions.

Figure S3 (related to Figure 2) (**A**) Torin-1 treatment specifically abolished uORF-mRNA loading on polysomes in response to auxin. uORF-RNA accumulation in polysomes in extracts prepared from 7 dag seedlings treated by NAA or Torin-1/ NAA for 8 h was analyzed by sqRT-PCR in each gradient fraction (**A**); quantifications see in (**B**). mRNAs were visualized by EtBr. sqRT-PCR was done in three replicates. The ribosomal profiles, distribution of 18S and 28S rRNAs within ribosomal profiles are shown. Graphs showing quantification of sqRT-PCR were corrected for polysome volume in NAA-, or Torin-1-treated ribosomal profiles. The highest value of each mRNA in polysomes from NAA/WT plants was set as 100%. Error bars indicate sd of the mean of three replicates. (**C**) Time course of either TOR or MPK3/MPK4/MPK6 phosphorylation in 7 dag seedlings before (0 h) and 4, 6, 8 and 10 h after transfer to medium with NAA analyzed by immunoblot with anti-mTOR-S2448-P antibodies and anti-phospho-*At*MPK3/4/6-P antibodies. LC loading control. Blots shown were repeated three times. (**D**) Protein synthesis from uORF-containing mRNAs is increased in response to auxin. Time course immunoblotting analyses of plants transgenic for ARF3:5'-UTR-3xGFP, ARF5:5'-UTR-3xGFP, ARF6:5'-UTR-3xGFP, ARF11:5'-UTR-3xGFP accumulation in 7 dag WT seedlings after transfer to the medium with either NAA, or Torin-1. GFP accumulation levels were followed by immunoblotting. The data shown are chosen from three representative independent blots.

Figure S4 (related to Figure 4) (**A**) *ARF3*, *ARF5*, *ARF6*, *ARF11*, *bZIP11* mRNA loading into polysomes is impaired in *TOR RNAi* plants. Polysomal distribution of uORF-mRNAs in extracts prepared from *TOR RNAi* seedlings treated (or not, Mock) with NAA or Torin-1 during 8 h. Distribution of mRNA was analyzed by sqRT-PCR (quantifications see in Fig. 4A) in each gradient fraction. mRNAs were visualized by EtBr. sqRT-PCR was done in three replicates. The ribosomal profiles, distribution of 18S and 28S rRNAs within ribosomal profiles are shown. (**B-E**) Accumulation of *ARF3, 5, 6* and *11*, but not *bZIP11* mRNAs is responsive to auxin in Torin-1-sensitive manner. qRT-PCR time-course analysis of transcript accumulation in WT seedlings after application of fresh medium with either non (**B**), or NAA (**C**), or Torin-1 (**D**); or in *TOR RNAi* seedlings after NAA application (**E**). Values, expressed in arbitrary units. Experiments were repeated three times.

Figure S5 (related to Figure 5) (**A**) *ARF3*, *ARF5*, *ARF6*, *ARF11*, *bZIP11* mRNA loading into polysomes is abolished in *eif3h-1* mutant plants. Polysomal distribution of uORF-mRNAs in extracts prepared from *eif3h-1* seedlings treated with NAA or Torin-1 during 8 h. Distribution of total mRNA was analyzed by sqRT-PCR in each gradient fraction (quantifications see in Fig. 5A) mRNAs were visualized by EtBr. sqRT-PCR was done in three replicates. The ribosomal profiles, distribution of 18S and 28S rRNAs within ribosomal profiles are shown. (**B**) Superimposition of polysomal profiles (relative to 80S peaks) obtained from extracts prepared from either WT, or *TOR RNAi*, or *eif3h-1* mutant seedlings treated with NAA (red) or Torin-1 (black).

Figure S6 (related to Figure 6) eIF3h interacts with S6K1 *in vivo*. (**A**) Schematic representation of TOR, the NTOR fused to Gal4 binding domain (BD-NTOR), BD-CTOR, BD-Raptor1B, BD-S6K1 and eIF3h fused to Gal4 activation domain (AD). TOR heat repeat domains (HR), FAT, FRB, PI3K and FATc domains are indicated. (**B**) Yeast two-hybrid analysis was carried out with AD or AD-eIF3h against BD-S6K1, BD-Raptor1B, BD-NTOR, BD-CTOR and BD. Two dilutions of the transformation mixture are shown. Experiments were done in triplicates and repeated at least three times.

Figure S7 (related to Figure 8) The eIF3h phosphorylation mimic associates with polysomes. Mesophyll protoplasts prepared from *eif3h-1* mutant seedlings were transformed with (or not, mock) the reporter plasmid encoding eIF3h, or eIF3h-S178D, or eIF3h-S178A. After 18 h of protoplast incubation, extracts were analyzed by sucrose gradient sedimentation. Polysomal profiles are presented. Distribution of eIF3h, eIF3h-S178D, or eIF3h-S178A in polysome gradient fractions was analysed by immunostaining using anti-eIF3h antibodies.