Supplemental Materials and methods

Construction of plasmids and baculoviruses for deletion mutants of r-eIF3

For r-eIF3(del-c), a FLAG-tag sequence was added to the C-terminus of the eIF3b coding region to construct pAcAB4-eIF3b-FLAG/eIF3l/eIF3g/eIF3i. Three baculoviruses expressing eIF3a-His + eIF3k, eIF3b-FLAG + eIF3g + eIF3i + eIF3l, and eIF3e + eIF3f + eIF3h + eIF3d were prepared.

For r-eIF3(del-d), eIF3a-His and eIF3c-FLAG cDNAs were cloned in pAcAB3(m) to produce pAcDB3-eIF3a-His/eIF3c-FLAG. eIF3e, eIF3f, eIF3h and eIF3k cDNAs were also cloned in pAcAB4(m) to produce pAcAB4-eIF3e/eIF3f/eIF3h/eIF3k. Three baculoviruses expressing eIF3a-His + eIF3c-FLAG, eIF3b + eIF3g + eIF3i + eIF3l, and eIF3e + eIF3f + eIF3h + eIF3k were prepared.

For r-eIF3(del-l), eIF3b, eIF3g and eIF3i cDNAs were cloned in pAcAB3(m) to generate pAcDB3-eIF3b/eIF3g/eIF3i. Three baculoviruses expressing eIF3a-His + eIF3c-FLAG + eIF3k, eIF3b + eIF3g + eIF3i, and eIF3d + eIF3e + eIF3f + eIF3h were prepared.

For r-eIF3(del-k), three baculoviruses expressing eIF3a-His + eIF3c-FLAG, eIF3b + eIF3g + eIF3i + eIF3l, and eIF3d + eIF3e + eIF3f + eIF3h were prepared.

For r-eIF3(del-g), eIF3b, eIF3i, eIF3k and eIF3l were cloned in pAcAB4(m) to generate pAcAB4-eIF3b/eIF3i/eIF3k/eIF3l. Three baculoviruses expressing eIF3a-His + eIF3c-FLAG, eIF3b + eIF3i + eIF3k + eIF3l, and eIF3d + eIF3e + eIF3f + eIF3h were prepared.

For r-eIF3(del-i), eIF3b, eIF3g, eIF3k and eIF3l were cloned in pAcAB4(m) to generate pAcAB4-eIF3b/eIF3g/eIF3k/eIF3l. Three baculoviruses expressing eIF3a-His + eIF3c-FLAG, eIF3b + eIF3g + eIF3k + eIF3l, and eIF3d + eIF3e + eIF3f + eIF3h were prepared.
For a complex comprising eIF3a, eIF3b, eIF3c, eIF3e, eIF3f and eIF3h, cDNAs for eIF3b, eIF3c, eIF3f and eIF3h were cloned in pAcAB4(m) to generate pAcAB4-eIF3b/eIF3c/eIF3f/eIF3h. Two baculoviruses expressing eIF3a-His + eIF3c-FLAG and eIF3b + eIF3e + eIF3f + eIF3h were prepared.

For a complex comprising eIF3a, eIF3b, eIF3e, eIF3f and eIF3h, cDNAs for eIF3e, eIF3f and eIF3h were cloned in pAcAB4(m) to generate pAcAB4-eIF3e/eIF3f/eIF3h. Two baculoviruses expressing eIF3a-His + eIF3b-FLAG and eIF3e + eIF3f + eIF3h were prepared.

**Mass spectrometric analysis**

Native or recombinant eIF3 was resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie brilliant blue (CBB). Protein bands were excised and digested with trypsin. The resulting peptides were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis. Monoisotopic peptide masses were assigned and used for data base searching ([http://www.matrixscience.com/search_form_select.html](http://www.matrixscience.com/search_form_select.html)).

**Antibodies**

An antibody to eIF3b was obtained from Santa Cruz Biotechnology. Antiserum to eIF3d was obtained by immunization of a rabbit with an N-terminal peptide (WGPCA VPEQFRDMPY) of the protein sequence. Antisera to eIF3g and eIF3k were obtained from rabbits immunized with His-eIF3g (full-length) and eIF3k (full-length),...
respectively. Antisera to eIF3a, eIF3c, eIF3e, eIF3f, eIF3h, eIF3i, eIF3j and eIF3l were raised in rabbits injected with glutathione-s-transferase (GST)-eIF3a (amino acids 997-1128), GST-eIF3c (amino acids 1-101), GST-eIF3e (amino acids 29-140), GST-eIF3f (full-length), GST-eIF3h (full-length), GST-eIF3i (full-length), GST-eIF3j (full-length) and GST-eIF3l (amino acids 2-140), respectively. Western blotting was performed using a standard procedure and protein bands were visualized and quantified by LAS 1000 (Fuji).

siRNA experiments

HeLa cells were transfected with siRNA against eIF3g (sense strand: 5’-GGAGGUCAUCAACGGAAAC, antisense strand: 5’-GUUUCCGUUGAUGACCUCC) using HiPerFect (QIAGEN) according to manufacture’s instructions and cultured for 2 days. A control siRNA was purchased from Ambion. Transfected cells (ten to twenty 100-mm dishes for each transfection) were collected and frozen at –80°C. Frozen cells were suspended in a 10-times volume of a sucrose buffer (0.25 M sucrose, 20 mM HEPES, pH 7.5, 10 mM KCl, 2 mM magnesium acetate, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, Complete EDTA-free [Roche]) containing 0.25% Triton X-100, kept on ice for 10 min and centrifuged at 10 500 × g for 10 min. An aliquot of the supernatant was analyzed by Western blotting with anti-eIF3i, anti–eIF3g or anti-eIF3c antiserum using LAS 1000 (Fuji). The rest of the
supernatant was centrifuged at 26000 rpm for 16 h in a Ti-70 rotor (Beckman). The pellet (a crude ribosomal fraction) was dissolved in 0.8 ml of the sucrose buffer without a detergent, and then 200 µl 3 M KCl was added. Following incubation on ice for 1 h, the sample was centrifuged at 38000 rpm for 16 h in a Ti-70 rotor (Beckman). The supernatant (a salt-wash of ribosomes) was dialyzed against a gradient buffer (0.35 M KCl, 20 mM HEPES, pH 7.5, 0.1 mM EDTA, 5 mM 2-mercaptoethanol) and layered on a 10-30% sucrose gradient prepared in the same buffer. Centrifugation was performed in an SW41 rotor (Beckman) at 38 000 rpm for 24 h. Every fraction (500 µl each) from the top (fr. 1) to the bottom (fr. 23) was mixed with 100 µl trichloroacetic acid (100%), kept on ice overnight, and then centrifuged at 12000 rpm for 15 min. The pellets were resolved on SDS-PAGE followed by Western blotting.

To monitor the rate of protein synthesis, HeLa cells transfected with siRNA were metabolically labeled with $^{35}$S methionine for 30 min. Cell extracts were resolved by SDS-PAGE, and $^{35}$S-labeled products were quantified using BAS 2000 (Fuji).
Figure S1

Non-conserved subunits are required for stable association of eIF3c with eIF3a and eIF3b. Evolutionarily conserved subunits (eIF3a-His, eIF3b, eIF3c-FLAG, eIF3g and eIF3i) or 11 subunits (the five conserved subunits plus six non-conserved subunits) were expressed in insect cells. An aliquot of the cell extract was analyzed by Western blotting with anti-eIF3a, eIF3b or eIF3c (left panel). Proteins were purified from remaining cell extracts by anti-FLAG chromatography with a buffer containing 100 mM KCl. Purified proteins were analyzed by Western blotting (right panel).