eIF3 targets cell-proliferation messenger RNAs for translational activation or repression

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Regulation of protein synthesis is fundamental for all aspects of eukaryotic biology by controlling development, homeostasis and stress responses6–8. The 13-subunit, 800-kilodalton eukaryotic initiation factor 3 (eIF3) organizes initiation factor and ribosome interactions required for productive translation9. However, current understanding of eIF3 function does not explain genetic evidence correlating eIF3 deregulation with tissue-specific cancers and developmental defects4. Here we report the genome-wide discovery of human transcripts that interact with eIF3 using photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP)5. eIF3 binds to a highly specific program of messenger RNAs involved in cell growth control processes, including cell cycling, differentiation and apoptosis, via the mRNA 5’ untranslated region. Surprisingly, functional analysis of the interaction between eIF3 and two mRNAs encoding the cell proliferation regulators c-JUN and BTG1 reveals that eIF3 uses different modes of RNA stem–loop binding to exert either translational activation or repression. Our findings illuminate a new role for eIF3 in governing a specialized repertoire of gene expression and suggest that binding of eIF3 to specific mRNAs could be targeted to control carcinogenesis.

Extensive genetic evidence implicates eIF3 in other functions in translation outside of its general role as a protein scaffold for the formation of initiation complexes. Mutation or inactivation of eIF3 subunits results in developmental defects in Caenorhabditis elegans and zebrafish10,11. Furthermore, analyses of human tumours reveal that overexpression of eIF3 is linked to diverse cancers, including breast, prostate and oesophageal malignancies12,13. The integral role of eIF3 during cellular differentiation, growth and carcinogenesis suggests that eIF3 might drive specialized translation. Consistent with this hypothesis, translation of hepatitis C virus RNA occurs through essential interactions between eIF3 and a structured internal ribosome entry site (IRES) element in the viral genome, indicating the feasibility of translation regulation being driven by distinct cellular eIF3–mRNA contacts8.

To identify candidate transcripts regulated through direct interactions with eIF3, we first used a genome-wide approach to determine the eIF3 RNA-binding targets in human 293T cells. Because eIF3 is composed of 13 subunits (eIF3a–m), we adapted a 4-thiouridine PAR-CLIP approach to allow analysis of a large multimeric complex, with isolation of individual subunit–RNA libraries (Fig. 1a). As overexpression of single eIF3 subunits can alter complex assembly8, we optimized immunoprecipitation of the full endogenous eIF3 complex using an antibody that recognizes the eIF3b subunit (Fig. 1b). High-salt washes were used to ensure removal of potentially contaminating translation factors, such as eIF4G or the small ribosomal subunit (Fig. 1c). After RNase digestion, separation of crosslinked eIF3–RNA complexes by denaturing gel electrophoresis demonstrated that four of the thirteen subunits crosslink directly to RNA (Fig. 1d), identified by mass spectrometry as eIF3a, b, d and g (Extended Data Fig. 1).

For each subunit, separate complementary DNA libraries were generated from the isolated crosslinked RNAs and deep sequenced using Illumina technology. Sequenced reads from three biological replicates were mapped to the genome and grouped into eIF3-binding sites by using the cluster-finding tool PARalyzer10. Read clusters were found in 479 unique genes, with eIF3a, b, d and g crosslinking to 328, 264, 356 and 352 transcripts, respectively (Supplementary Tables 1 and 2). The limited number of interacting genes supports capture of specific eIF3–RNA contacts, as these targets compromise only ~3% of total expressed transcripts (Extended Data Fig. 2). As a further control, we do not see crosslinking...
with distinct combinations of eIF3a, b, d and g subunits (Fig. 2c). To validate the RNAs identified by PAR-CLIP, we performed eIF3 immunoprecipitation in the absence of crosslinking. We detected eIF3–RNA interactions for five top candidate genes using polymerase chain reaction with reverse transcription (RT–PCR); whereas a negative control mRNA, the PSMB6 transcript, was not immunoprecipitated (Fig. 2d).

In eukaryotic protein synthesis, the 5′ UTR of mRNA is thought to be the major site of translation regulation. In agreement with identifying translation regulation roles of specific eIF3–mRNA interactions, the eIF3-binding sites predominantly mapped to the 5′ UTR (~70%) (Fig. 2e). To examine the impact of transcript-specific engagement of eIF3 on translational control, we focused on two genes with an eIF3-binding site in the 5′ UTR, c-JUN and B-cell translocation gene 1 (BTG1) (Fig. 3a, b). c-JUN is a member of the immediate early response transcription factor AP1 and a positive mitotic regulator. In contrast, BTG1 acts as a negative regulator of proliferation and its expression induces cellular differentiation. Because of the opposing effects of c-JUN and BTG1 on cellular growth, we wanted to understand why eIF3 would interact with both mRNAs. We constructed luciferase reporters containing the 5′ UTR of c-JUN or BTG1 with or without the eIF3 crosslinking site identified by PAR-CLIP (Fig. 3c). Deletion of the crosslinking site from the 5′ UTR of c-JUN abolished translation of mRNAs transfected into cells, indicating that eIF3 binding is required for efficient translation (Fig. 3d). In stark contrast, BTG1 translation was highly upregulated when the eIF3-binding site was removed from the mRNA (Fig. 3e).

Furthermore, treatment of 293T cell in vitro translation extracts with m7G cap analogue inhibited translation of both c-JUN and BTG1 luciferase reporter mRNAs, demonstrating that eIF3-dependent translation regulation of these transcripts is cap-dependent and thus distinct from viral IRES-like mechanisms (Fig. 3f, g). These results demonstrate that eIF3 can act as both a translation activator and repressor of specific cellular mRNAs.

To understand how eIF3 binding to mRNA leads to opposing translation phenotypes, we next identified the full RNA elements for eIF3 recognition in the c-JUN and BTG1 mRNAs. While PAR-CLIP marks the localized vicinity of eIF3 in the 5′ UTR, eIF3 interaction could occur either through recognition of a linear sequence or in the context of RNA secondary structure. Using selective 2′-hydroxyl acylation analysed by primer extension (SHAPE), we experimentally determined the secondary structure around the eIF3-binding sites (Fig. 4a, d). For both c-JUN and BTG1, SHAPE revealed that the eIF3-binding sites map to structured

to highly abundant ribosomal RNAs, in agreement with biochemical and structural studies showing that eIF3 interacts primarily with the protein-rich face of the small ribosomal subunit.

The majority of RNAs contained a single eIF3-binding site, with a median cluster length of 25 nucleotides (Fig. 2a, b). These RNAs interact with eIF3 and the 5′ UTR of mRNA is thought to be the major site of translation regulation. In agreement with identifying translation regulation roles of specific eIF3–mRNA interactions, the eIF3-binding sites predominantly mapped to the 5′ UTR (~70%) (Fig. 2e). To examine the impact of transcript-specific engagement of eIF3 on translational control, we focused on two genes with an eIF3-binding site in the 5′ UTR, c-JUN and B-cell translocation gene 1 (BTG1) (Fig. 3a, b). c-JUN is a member of the immediate early response transcription factor AP1 and a positive mitotic regulator. In contrast, BTG1 acts as a negative regulator of proliferation and its expression induces cellular differentiation. Because of the opposing effects of c-JUN and BTG1 on cellular growth, we wanted to understand why eIF3 would interact with both mRNAs. We constructed luciferase reporters containing the 5′ UTR of c-JUN or BTG1 with or without the eIF3 crosslinking site identified by PAR-CLIP (Fig. 3c). Deletion of the crosslinking site from the 5′ UTR of c-JUN abolished translation of mRNAs transfected into cells, indicating that eIF3 binding is required for efficient translation (Fig. 3d). In stark contrast, BTG1 translation was highly upregulated when the eIF3-binding site was removed from the mRNA (Fig. 3e).

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Figure 2 | Analysis and validation of eIF3 PAR-CLIP-derived binding sites. a, Length distribution of PAR-CLIP clusters. nt, nucleotides. b, Distribution of number of PAR-CLIP clusters per gene. c, Distribution of PAR-CLIP targets among different combinations of eIF3 subunit crosslinking. d, Validation of PAR-CLIP targets by eIF3 immunoprecipitation and RT–PCR. eIF3 immunoprecipitation (IP) was performed using an anti-eIF3b antibody as in Fig. 1b. As negative controls, the immunoprecipitation was performed with isotype-matched immunoglobulin G (IgG) or anti-haemagglutinin tag (HA) antibody. e, Distribution of eIF3 crosslinking sites along mRNAs and in other classes of RNAs. CDS, coding sequence; Misc., miscellaneous.

Figure 3 | eIF3 is a positive and negative transcript-specific translational regulator. a, b, eIF3 PAR-CLIP cluster in the 5′ UTR of c-JUN mRNA (a) or BTG1 mRNA (b). Reads mapped are shown along the respective genes. c, Schematic of c-JUN and BTG1 5′ UTR-luciferase reporter mRNAs. The eIF3 PAR-CLIP cluster is nucleotide positions 181–214 for the c-JUN transcript (GenBank accession NM_002228) and positions 105–187 for the BTG1 transcript (GenBank accession NM_001731). WT, wild type. d, e, Luciferase activity in cells transfected with mRNAs containing the c-JUN (d) or BTG1 (e) 5′ UTR with or without deletion of the eIF3 crosslinking site. f, g, Luciferase activity in vitro from mRNAs driven by the c-JUN (f) or BTG1 (g) 5′ UTR, with or without competitor m7G cap analogue. The results of d–g are given as the mean ± standard deviation (s.d.) of three independent experiments, each performed in triplicate.
RNA regions corresponding to conserved stem–loops (Extended Data Fig. 3). For the c-JUN mRNA element, we investigated the importance of secondary structure in eIF3 recognition by mutating base-pairing interactions of five nucleotides in the stem while leaving the cross-linking site intact (Fig. 4b). eIF3 directly bound to the c-JUN stem–loop but not the mutated stem–loop, as determined by native agarose gel electrophoresis with radiolabelled RNA and recombinant or native eIF3 (Fig. 4b and Extended Data Fig. 4). Furthermore, the same mutations in the c-JUN luciferase reporter mRNA led to the identical trans-activation (Extended Data Fig. 6a). The combination of these targets may represent a gene program that supports overactive cell proliferation during eIF3-related malignancies. In support of this, our results demonstrate that eIF3 acts as a positive translational regulator of c-JUN, which is a proto-oncogene required for RAS-mediated transformation; a negative regulator of BTG1, of which genomic deletions are found in 9% of B-cell precursor acute lymphoblastic leukaemias; and a proto-oncogene required for RAS-mediated transformation; and a negative regulator of BTG1, of which genomic deletions are found in 9% of B-cell precursor acute lymphoblastic leukaemias.

Although misregulation of eIF3 levels is implicated in carcinogenesis, it was previously unknown if eIF3 activities lead to these cell growth alterations. Gene ontology analysis of the PAR-CLIP results establish direct binding of eIF3 to RNA targets enriched in cancer-associated cell growth regulation pathways, such as apoptosis, cell cycling and differentiation (Extended Data Fig. 6a). The combination of these targets may represent a gene program that supports overactive cell proliferation during eIF3-related malignancies. In support of this, our results demonstrate that eIF3 acts as a positive translational regulator of c-JUN, which is a proto-oncogene required for RAS-mediated transformation; and a negative regulator of BTG1, of which genomic deletions are found in 9% of B-cell precursor acute lymphoblastic leukaemias. Furthermore, circumventing eIF3 translational control by knockdown of c-JUN or overexpression of BTG1 decreases cell invasiveness of H1299 human lung cancer cells, which overexpress eIF3a.

Figure 4 | Opposing translation phenotypes are driven by different modes of eIF3–mRNA binding. a, SHAPE-based secondary structure of the c-JUN 5′ UTR surrounding the eIF3 PAR-CLIP site. Nucleotides are colour-coded by their SHAPE reactivities, with higher reactivity reflecting single-stranded behaviour and non-reactivity indicating base pairing between nucleotides. b, Representative native gel shifts showing a specific and binary interaction between recombinant eIF3 and the wild-type (WT) c-JUN stem–loop (SL) structure but not the mutated stem–loop. c, Luciferase (Luc) activity in vitro of mRNAs driven by the c-JUN 5′ UTR containing stem–loop mutations. Mut SL, mutant stem–loop. d, SHAPE-based secondary structure of the BTG1 5′ UTR surrounding the eIF3 PAR-CLIP site. e, Luciferase activity in vitro from mRNAs driven by a PSMB6 5′ UTR–BTG1 stem–loop chimaera. Rev SL, transversed stem–loop. The results of c and e are given as the mean ± s.d. of three independent experiments, each performed in triplicate. f, g, Representative images of the effect of siRNA-mediated knockdown of c-JUN (f) or BTG1 overexpression (g) on Matrigel invasion by H1299 cells. As a control, cells were transfected with a non-targeting siRNA (i) or empty vector (g). Quantification of cell migration is presented in Extended Data Fig. 6. The results of f and g are representative of three independent experiments, each performed in duplicate.
upon eIF3 overexpression, leading to loss of correct translational control of cell growth and eventual malignancy.

Although it is surprising that eIF3 can act as both a repressor and activator of translation, analogous contrasting functions have been found with other multi-protein complexes. For example, the RNA polymerase II regulation complex Mediator consists of at least 30 proteins in humans. It directs either transcription activation or repression, dependent on promoter sequence, gene-specific regulatory proteins, and altered phosphorylation states of subunits23. Intriguingly, more than 25 posttranslational modifications have been detected on eIF3, with a number of them at stoichiometric level24,25, and eIF3 association with other translation regulatory proteins such as the helicase eIF4B is regulated by mitogenic signalling26. Furthermore, modelling of the eIF3 subunits, except for eIF3d, reveals that the crosslinked subunits form a nexus in a distal region of eIF3 positioned near the mRNA entry tunnel (Extended Data Fig. 7)14. As revealed that the crosslinked subunits form a nexus in a distal region of eIF3 translation through direct binding to defined RNA structural elements. Both an activator and repressor of cap-dependent transcript-specific control, in which, in addition to this general function, eIF3 can act as an activator of protein arginine methyl transferase I. J. Cell Biol. 164, 175–184 (2004).

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Supplementary Information is available online in the version of the paper.

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METHODS

Cells and transfections. Human 293T cells were maintained in DMEM (Invitrogen) with 10% FBS (Tissue Culture Biologicals), H1299 cells were maintained in RPMI-1640 ATCC-formulated (ATCC) with 10% FBS. IMR90 cells were maintained in Eagles MEM (ATCC) with 10% FBS. RNA transfections were performed using TransIT-mRNA reagent (Mirus), with the following modifications to the manufacturer’s protocol. Twenty-four hours before transfection, 293T cells were seeded into opaque 96-well plates to be at ~80% confluence at the time of transfection. For each well, 9 μl of pre-warmed OptiMEM (Invitrogen) was mixed with 90 ng of RNA, 0.27 μl of Boost reagent and 0.27 μl of TransIT-mRNA reagent. Reactions were incubated for 3 min at room temperature, added drop-wise to the well, and luciferase activity was assayed 18 h after transfection. Plasmid transfections were performed using Lipofectamine 2000 (Invitrogen), according to the manufacturer’s protocol, and Matrigel or western blot assays were performed 48 h after transfection. siRNA transfection was performed using Lipofectamine 2000, with the following modifications to the manufacturer’s protocol. Forty-eight hours after transfection, cells were split into new 6-well plates to be at ~70% confluence 24 h after seeding. These cells were then transfected for a second time with siRNA, and harvested for Matrigel or western blot assays 48 h after the second transfection.

Plasmids and siRNAs. To generate the c-Jun and BTG1 5′ UTR luciferase reporter plasmids, sections of the 5′UTR were first amplified from human cDNA. These were then subcloned downstream of a 5′ UTR luciferase reporter construct. The 5′UTR reporters were then transiently co-transfected with the luciferase reporter plasmid, pGL4.10, and the pRL-CMV reference plasmid (Promega). The cell suspension was incubated on ice for 10 min, passed through an 18G needle and the pellet was resuspended in three volumes of NP40 lysis buffer (50 mM HEPES-KOH pH 7.5, 0.5 mM KCl, and 1% Nonidet P-40) and the reactions were preincubated at 30°C for 1 h, after which luciferase activity was assayed.

ELF3 purification and native agarose gel electrophoresis. Reticulocyte ELF3 was expressed and purified from Escherichia coli and native ELF3 was purified from HeLa cells as previously described22. The gel shift protocol was adapted from previously described protocols19,24. A 0.7% agarose gel was prepared using Agarose Type 1B (Sigma A0576) in buffer consisting of 1× TBE supplemented with 75 mM KCl and gel and buffer were pre-cooled to 4°C. For each gel shift, 2 μl water, 1 μl of 5× Binding Buffer (125 mM Tris-HCl pH 7.5, 25 mM Mg(OAc)2, 350 mM KCl, 0.5 mM CaCl2, 0.5 mg ml−1 BSA, 10 mM TCEP), 1 μl labelled RNA and 1 μl of purified elf3 or protein buffer were added, in the listed order, and incubated at 25°C for 30 min. One microlitre of room temperature 6× non-denaturing loading dye (40% w/v sucrose, with xylene cyanol and bromophenol blue) was added to the reactions and these were loaded on the agarose gel. The gel was run for 1 h at 40 V with 4°C, buffer was replaced with fresh cold buffer, and the gel was run for another hour at 40 V. The gel was placed on top of positively charged nylon membrane with four pieces of Whatman filter underneath, covered in saran wrap, and dried for 1 h at 75°C on a pre-heated gel drier. The gel was imaged using a phosphomager.

SHAPE mapping of RNA structure. The SHAPE protocol was adapted from a previously described protocol2. Each RNA folding reaction contained 1 μg of RNA, 1.5 μl 5× annealing buffer (500 mM HEPES-KOH pH 8.0, 250 mM KCl, 12.5 mM MgCl2), 10% DMSO, and water to make the total reaction volume 9 μl. RNAs were incubated at 65°C for 5 min, ice for 5 min, and then at 25°C for 5 min. For each tube, 1 μl of 100% dimethylsulfoxide (DMSO) or 1 μl of 800 mM benzoyl cyanide (Sigma) was added, and the reaction was mixed by pipetting three times. The RNAs were immediately recovered by ethanol precipitation. Purified RNA was dissolved in 9 μl of 0.5× TE buffer (5 mM Tris, 0.5 mM EDTA, pH 8.0). Three microlitres of 0.3 μM NED- or VIC-labelled primers were added to the modified and unmodified reactions, respectively. For sequencing reactions, 1 μg of RNA in 1 μl was mixed with 8 μl of 0.5× TE buffer, and 3 μl of 0.3 μM FAM- or PET-labelled primers were added to each tube with 1 μl of 10 mM ddATP or ddTTP. To each tube, 7 μl of reverse transcription buffer (250 mM KCl, 167 mM Tris-HCl pH 8.3, 1.67 mM MgCl2, 0.3 mM dNTPs, 17 mM DTT and 10 mM MgCl2) was added and the reactions were prewarmed to 52°C for 1 min. One microlitre of Superscript III (Invitrogen) was added and the tubes were incubated at 52°C for 50 min, 65°C for 5 min, and then put on ice. The RNA was hydrolysed by adding 0.5 μl 10% NaOH, heating to 95°C for 3 min, put on ice, and then neutralized by adding 0.33 μl of 12.1 M HCl. cDNAs were recovered by ethanol precipitation and resuspended in 11 μl of deionized formamide. Fragment analysis was performed using an Applied Biosystems 3730XL DNA Analyzer, and raw traces were analysed using Shapfinder software6.

Matrigel invasion assay. Matrigel assays were performed using Corning BioCoat Matrigel invasion chambers according to the manufacturer’s protocol. Twenty-four hours after seeding the invasion chambers, invaded cells were fixed with 70% ethanol and stained with crystal violet before imaging.

PAR-CLIP. Three biological replicates of PAR-CLIP were performed as previously described23, with some modifications. For each experiment, 40–50 150 mm plates of 293T cells were seeded to be at ~90% confluence during crosslinking. Fourteen hours before crosslinking, 4-thiouridine (Sigma) was added to the media to a final concentration of 100 μM. For crosslinking, the cells were washed with cold PBS and then the plates were irradiated on ice with 0.15 cm−2 of UV 365 nm light. The cells were scraped into PBS, pelleted by centrifugation at 1,000 g for 5 min at 4°C, and the pellet was resuspended in three volumes of NP40 lysis buffer (50 mM HEPES-KOH, 150 mM NaCl, 0.5% Nonidet P-40 alternative, 0.5 mM DTT, 1 Complete EDTA-free Protease Inhibitor Cocktail tablet per 50 ml of buffer). The cell suspension was incubated on ice for 10 min, passed through an 18G needle five times, and centrifuged at 13,000g for 15 min at 4°C. The lysate was filtered through a 0.2 μm membrane syringe filter and RNAs were lightly digested by treatment with RNase T1 (Thermo Scientific) at a final concentration of 0.05 μU ml−1 for 15 min at room temperature. For each plate, 5 μl of Dynabeads (Invitrogen) and 10 μl of anti-EF3b antibody (Bethyl A301-761A) were prepared by washing the beads once with PBS and 0.2% Tween-20, and then allowing the antibody to bind to the beads in PBS and 0.2% Tween-20 by rotating at room temperature for 15 min. The antibody bound beads were added to the lysates and the immunoprecipitation was rotated at 4°C for 2 h.

The beads were collected and washed three times in high-salt NP40 wash buffer (50 mM HEPES-KOH pH 7.5, 500 mM KCl, 0.5% Nonidet P-40 alternative, 0.5 mM DTT, 1 Complete EDTA-free Protease Inhibitor Cocktail tablet per 50 ml of buffer). One bead volume of NP40 lysis buffer and 50 μl U−1 Rnase T1 was added to the beads and incubated for 16 min at room temperature. Beads were washed three times in high-salt NP40 wash buffer and resuspended in one bead volume of Buffer 3 (NEB) with 0.5 μU ml−1 Calf Intestinal Phosphatase (NEB). The reaction was incubated at 37°C for 10 min, and beads were washed twice in phosphate wash buffer (50 mM HEPES-KOH pH 7.5, 20 mM EGTA, 0.5% w/v Nonidet P-40 alternative) and twice in PNK buffer without DTT (50 mM Tris-HCl pH 7.5, 50 mM NaCl, 10 mM MgCl2). Beads were resuspended in one bead volume of PNK buffer with 0.5 μM [γ-32P]-ATP and 1 μl U−1 T4 PNK (NEB), and incubated for 20 min at 37°C. One-hundred micromolar nonradioactive ATP was added and the reaction was incu-
bated for 5 min at 37 °C, and then beads were washed five times with PNK buffer without DTT. SDS–PAGE loading dye (50 mM Tris–HCl pH 6.8, 100 mM b-mercaptoethanol, 2% w/v SDS, 10% v/v glycerol, 0.1% bromophenol blue) was added to the beads, the sample was boiled for 5 min, and the sample was loaded onto a Bis–Tris 4–12% Bis–Tris gel (Novex) and electrophoresed in MOPS buffer (2.5 mM MOPS, 2.5 mM Tris base, 0.005% w/v SDS, 1 mM EDTA). As a size standard, native elF3 was loaded onto the same gel.

The gel was imaged using a phosphorimager, a printed image was aligned to the gel, and the complexes were excised and electroeluted in a D Tube Dialyzer Midi (Millipore) for 2.5 h at 150 V, at 4 °C. The protein was digested with 1.2 mg ml−1 Proteinase K (Roche) in Proteinase K buffer (50 mM Tris–HCl pH 7.5, 75 mM NaCl, 6.25 mM EDTA, 1% w/v SDS) for 30 min at 37 °C. The RNA was isolated by phenol-chloroform extraction and ethanol precipitation, and small RNA libraries were prepared using a standard protocol17. The cDNA libraries were sequenced on an Illumina HiSeq 2000.

Mass spectrometry. Protein samples were prepared alongside the sequencing samples used for RNA library preparation, using five plates and substituting nonradioactive ATP during the T4 PNK labelling step. The samples were run on the same gel as the radiolabelled PAR-CLIP samples and cut out using the phosphorimager printout as a guide. Mass spectrometry samples were prepared by in-gel tryptic digestion9 and peptides were identified by liquid chromatography–mass spectrometry (LC-MS).

Denaturing immunoprecipitation. The denaturing immunoprecipitation was performed using the PAR-CLIP protocol, with the following alterations. Five plates were used for each sample and, after crosslinking, one volume of NP40 lysis buffer was added and the sample was incubated on ice for 10 min. The lysate was clarified by centrifugation, the supernatant was transferred to a new tube, and one volume of 2× SDS lysis buffer (10% w/v SDS, 100 mM Tris–HCl pH 7.4, 10 mM EDTA, 20 mM DTT) was added. The sample was boiled for 5 min, cooled on ice, and then diluted at least tenfold with nondenaturing lysis buffer (1/5% Triton-X 100, 50 mM HEPES–KOH pH 7.5, 150 mM NaCl, 2 mM EDTA). Immunoprecipitation was performed using an anti-elF3d (Bethyl A301-758A) or anti-elF3g (Bethyl A301-757A) antibody. PAR-CLIP computational analysis. Raw Illumina reads were collapsed using fastx_collapser from FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and 3′ adapters were removed using Cutadapt (http://code.google.com/p/cutadapt/). Reads shorter than 15 nucleotides were discarded. To remove processed reads that align to repeat elements, reads were mapped using Bowtie2 to the hg19 RepeatMasker track from the UCSC table browser, and unmapped reads were retained. Retained reads were mapped to the hg19 reference genome, allowing for up to two mismatches in alignment. PARalyzer28 was used to identify read clusters, or elF3 crosslinking sites, from the UCSC table browser, and unmapped reads were retained. Retained reads were mapped to the human hg19 genome using TopHat2 and the Genome (v1) annotation. FPKM was calculated using a python script, and the average FPKM was calculated using the two biological replicates.

RNA immunoprecipitation and RT–PCR. Two 150 mm plates of 293T cells were lysed in three volumes of NP40 lysis buffer. Dynabeads were prepared with rabbit IgG (Cell Signaling 2729), rabbit anti-HA antibody (Invitrogen 71-5500) or rabbit anti-elF3b antibody (Bethyl A301-761A). The lysate was split into three parts, the different antibody–Dynabead mixtures were added, and the suspension was incubated for 2 h at 4 °C. The beads were washed four times with 150 mM NP40 wash buffer (50 mM HEPES-KOH pH 7.5, 500 mM KCl, 2 mM EDTA, 1% Nonidet P-40 alternative, 0.5 mM DTT), and bound RNAs were isolated by phenol-chloroform extraction and ethanol precipitation. cDNA was reverse transcribed using random hexamers and Superscript III, and PCR was performed using Phusion. The following oligonucleotides were used. RANGAP1-Forward, 5′-ACGGTCTGGAAGATAGATGG-3′; RANGAP1-Reverse, 5′-CGGAAAGGTTCCAAGGCCTC-3′; JUN-Forward, 5′-TGACTCAGGAAATGAGAAC-3′; JUN-Reverse, 5′-CCGGTCTGAGCTGGAT-3′; BTG1-Forward, 5′-CAGGAGTTCAGAGGAGAGAG-3′; BTG1-Reverse, 5′-TCATCCATTACGGCTGGTGTG-3′; PSMB3-Forward, 5′-GAATTCCTTCAGCCGGAGATG-3′; PSMB3-Reverse, 5′-TCCGGTATGGTGAAGCTTC-3′.

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Sun, C. et al. Functional reconstitution of human eukaryotic translation initiation factor 3 (eIF3). Proc. Natl Acad. Sci. USA 108, 20473–20478 (2011).
Extended Data Figure 1 | PAR-CLIP reveals eIF3a, b, d and g bind to RNA.

a, Mass spectrometry identification of trypsin-released peptides from RNA-crosslinked eIF3 subunits. Peptides identified by mass spectrometry are highlighted in pink.

b, c, Crosslinking and denaturing immunoprecipitation to validate subunit identification. As eIF3d and g co-migrate with eIF3l and e/f, respectively, subunit identification was validated by immunoprecipitation of individual proteins after crosslinking and treatment of lysates with SDS.
Extended Data Figure 2 | Analysis of eIF3 PAR-CLIP targets. a, Scatterplot of fragments per kilobase of exon per million reads (FPKM) of all mRNAs expressed in 293T cells. mRNAs that are eIF3 PAR-CLIP targets are highlighted in red. b, Scatterplot of correlation between mRNA expression and PAR-CLIP read coverage for mRNAs that are eIF3 PAR-CLIP targets. The simple linear regression line is plotted in blue, with the 95% confidence region shaded in grey.
Extended Data Figure 3 | Conservation of c-JUN and BTG1 eIF3-binding sites in primates and mammals. a, b. The eIF3-binding site is indicated in cyan. nt, nucleotides. a, c-JUN GenBank accessions are: human (NM_002228.3, Homo sapiens), chimpanzee (XM_513442.5, Pan troglodytes), gorilla (XM_004025880.1, Gorilla gorilla), orangutan (XM_002810763.3, Pongo abelii), rhesus macaque (XM_002750880.3, Callithrix jacchus), mouse (NM_010591.2, Mus musculus), cat (XM_006934825.1, Felis catus). b, BTG1 GenBank accessions are: human (NM_001731.2, Homo sapiens), chimpanzee (XM_509262.3, Pan troglodytes), orangutan (XM_002823578.2, Pongo abelii), rhesus macaque (NM_001266672.1, Macaca mulatta), marmoset (XM_002752814.3, Callithrix jacchus), mouse (NM_007569.2, Mus musculus), cat (XM_006933950.1, Felis catus), cow (NM_173999.3, Bos taurus).
Extended Data Figure 4 | Interactions between native and recombinant eIF3 and the c-JUN and BTG1 RNA stem–loops. a, Coomassie blue staining of purified native HeLa eIF3 or recombinant eIF3, resolved by SDS–PAGE. b, Representative native agarose gel electrophoresis shows a specific and binary interaction between native (Nat) and recombinant (Rec) eIF3 and the wild-type (WT) c-JUN stem–loop structure, but not with the mutated stem–loop or the wild-type BTG1 stem–loop.
Extended Data Figure 5 | Luciferase activity of c-JUN and BTG1 mutants in cells. 

(a) Luciferase activity in 293T cells transfected with mRNAs containing the c-JUN 5’ UTR with a mutated stem–loop (a) or the PSMB6 5’ UTR-BTG1 stem–loop chimaera (b). Mut, mutant; Rev, transversed; SL, stem–loop; WT, wild type. The results are given as the mean ± s.d. of three independent experiments, each performed in triplicate.
Extended Data Figure 6 | Bypassing eIF3 translational control in H1299 cells reduces cell invasiveness. a, Functional classification of eIF3-bound RNAs. b, Representative western blot analysis of eIF3a expression levels in H1299 and IMR90 cells. GAPDH was detected as a loading control for normalized protein levels. c, Representative image of Matrigel invasion by H1299 or IMR90 cells. d, BTG1 protein levels after overexpression in H1299 cells. HSP90 was detected as a loading control. e, Matrigel invasion assay in H1299 cells after overexpression of BTG1. ORF, open reading frame. f, c-JUN protein levels after siRNA-mediated knockdown in H1299 cells. NT, non-targeting. g, Matrigel invasion assay in H1299 cells after knockdown of c-JUN. The results of e and g are given as the mean ± s.d. of three independent experiments, each performed in duplicate.
Extended Data Figure 7 | Schematic of eIF3 subunit localization on the small ribosomal subunit. The eIF3 subunits bound to RNA in the PAR-CLIP experiment, eIF3a, b and g, form a nexus in the distal eIF3 region. The location of eIF3d has not been assigned, and the schematic is adapted from ref. 14.