The EMBO Journal (2007) 26, 3373–3383 | © 2007 European Molecular Biology Organization | All Rights Reserved 0261-4189/07
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THE EMBO JOURNAL

Reconstitution reveals the functional core of mammalian eIF3

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Eukaryotic translation initiation factor (eIF)3 is the largest eIF (~650 kDa), consisting of 10–13 different polypeptide subunits in mammalian cells. To understand the role of each subunit, we successfully reconstituted a human eIF3 complex consisting of 11 subunits that promoted the recruitment of the 40S ribosomal subunit to mRNA. Strikingly, the eIF3g and eIF3i subunits, which are evolutionarily conserved between human and the yeast *Saccharomyces cerevisiae* are dispensable for active mammalian eIF3 complex formation. Extensive deletion analyses suggest that three evolutionarily conserved subunits (eIF3a, eIF3b, and eIF3c) and three non-conserved subunits (eIF3e, eIF3f, and eIF3h) comprise the functional core of mammalian eIF3.

The EMBO Journal (2007) **26,** 3373–3383. doi:10.1038/sj.emboj.7601765; Published online 21 June 2007
Subject Categories: proteins
Keywords: eIF; eIF3; reconstitution; translation; translation initiation

Introduction

Eukaryotic protein synthesis requires the participation of translation initiation factors that mediate mRNA binding to the 40S ribosomal subunit. Ribosome binding is facilitated by the cap structure (m7GpppN, where N is any nucleotide) at the 5′ end of all cellular mRNAs (except organellar mRNAs). The mRNA cap structure initially binds to eukaryotic initiation factor (eIF) 4F, which consists of the cap binding protein eIF4E, an RNA helicase eIF4A, and a modular scaffold protein eIF4G. eIF4F, in conjunction with eIF4B, disrupts mRNA secondary structure in its 5′-untranslated region and promotes ribosome binding. The 40S ribosomal subunit, in a complex with eIF3, eIF1, eIF1A, and eIF2-GTP-Met-tRNAi^{Met}, binds at or near the cap structure and scans the 5′-untranslated region in search of the initiator AUG codon (Gingras *et al*, 1999; Hinnebusch, 2000; Raught *et al*, 2000). eIF3 is the largest translation initiation factor (~650 kDa) (Behlke *et al*, 1986), and consists of 10–13 different polypeptide subunits in mammalian cells (Hershey and Merrick, 2000; Unbehaun *et al*, 2004).

eIF3 is a moderately abundant translation initiation factor, with 0.5–1 molecules/ribosome in HeLa cells and rabbit reticulocyte lysate (Meyer *et al*, 1982; Mengod and Trachsel, 1985). eIF3 plays many functions in initiation complex formation. It interacts with eIF1, eIF5, eIF4B and eIF4G, and the direct interaction between eIF3 and eIF4G may serve as a bridge between the 40S ribosomal subunit and eIF4F-bound mRNA (Hershey and Merrick, 2000). eIF3 stabilizes the binding of the eIF2-GTP-Met-tRNAi^{Met} ternary complex to the 40S subunit (Chaudhuri *et al*, 1999), and, reciprocally, binding of the ternary complex to the 40S subunit enhances eIF3 binding to the ribosome (Kolupaeva *et al*, 2005). Even in the absence of the ternary complex, the presence of RNA can promote the binding of eIF3 to the 40S subunit (Kolupaeva *et al*, 2005). Hepatitis C virus (HCV) is proposed to take advantage of this property of eIF3 to correctly position the viral internal ribosome entry site (IRES) on the 40S ribosome (Siridechadilok *et al*, 2005). Association of eIF3 with the ribosome is stabilized by a loosely bound eIF3 subunit, eIF3j (Fraser *et al*, 2004). eIF3j is not associated with the eIF3 complex in quiescent T lymphocytes, but upon mitogenic stimulation joins the other eIF3 subunits, promoting eIF3 binding to 40S ribosomes. This may contribute to increased translation rates during T-lymphocyte activation (Miyamoto *et al*, 2005). Protein kinases such as mTOR and S6K1 also regulate translation initiation by associating with eIF3 in response to nutrients, energy sufficiency, hormones, and mitogenic agents (Holz *et al*, 2005; Harris *et al*, 2006). The 20S proteasome specifically cleaves eIF3a, the largest subunit of eIF3, and differentially affects translation of different mRNAs (Baugh and Pilipenko, 2004). Taken together, these findings clearly establish that eIF3 is a pivotal player in translational control (Hinnebusch, 2006).

The function of each subunit of eIF3 has been investigated in *Saccharomyces cerevisiae*. In budding yeast, eIF3 comprises only five subunits, TIF32 (eIF3a), PRT1 (eIF3b), NIP1 (eIF3c), TIF35 (eIF3g), and TIF34 (eIF3i) (Asano *et al*, 1998; Phan *et al*, 1998), all of which are conserved in mammalian eIF3. Deletion or mutation of eIF3a (Valasek *et al*, 1998; Vornlocher *et al*, 1999), eIF3b (Danaie *et al*, 1995; Phan *et al*, 1998), eIF3c (Greenberg *et al*, 1998), eIF3g (Hanachi *et al*, 1999), or eIF3i (Naranda *et al*, 1997; Verlhac *et al*, 1997) in yeast leads to a vast reduction in protein synthesis, suggesting that each of these five subunits is required for eIF3 integrity. However, a biochemical study using yeast extract suggested that a subcomplex comprising the large three subunits eIF3a, eIF3b, and eIF3c supported translation initiation, and that the small two subunits, eIF3g and eIF3i were dispensable for translation at least *in vitro* (Phan *et al*, 2001).

Analysis of mammalian eIF3 has been difficult due to the complexity of eIF3 and to a lack of genetic tools. Because the non-conserved subunits of mammalian eIF3 are not only

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Received: 5 March 2007; accepted: 24 May 2007; published online: 21 June 2007

binding partners but are integral components of the eIF3 complex (Hershey *et al*, 1996; Unbehaun *et al*, 2004), the structure of the mammalian eIF3 is likely to be considerably different from the yeast eIF3.

As a major step toward elucidating the structure of this multisubunit translation initiation factor and the molecular mechanisms of the mammalian eIF3-mediated translational controls, we reconstituted the human eIF3 by coexpressing 11 human subunit proteins. Remarkably, deletion studies showed that a recombinant eIF3 complex lacking two evolutionarily conserved subunits and three non-conserved subunits is able to support translation initiation and thus constitutes the core of mammalian eIF3.

Results

Reconstitution of human eIF3

Mammalian eIF3 has been reported to consist of 10–13 subunits (Hershey *et al*, 1996; Asano *et al*, 1997a; Unbehaun *et al*, 2004), so we wished to first determine the composition of native eIF3 (n-eIF3) purified from mouse Krebs-2 cells. Purified n-eIF3 was resolved by SDS–PAGE (Figure 1A), and each band was identified by MALDI-TOF/MS analysis. Eleven proteins were identified as comprising n-eIF3 (Figure 1A): p170 (eIF3a), p116 (eIF3b), p110 (eIF3c), p69 (eIF3l), p66 (eIF3d), p48 (eIF3e), p47 (eIF3f), p44 (eIF3g), p40 (eIF3h), p36 (eIF3i), and p28 (eIF3k); notably, eIF3j (p35) was absent. Western blot analysis with subunit-specific antisera confirmed the identity of all of the eIF3 complex subunits identified by mass spectrometry (Figure 1B). Anti-eIF3j antiserum failed to detect eIF3j in n-eIF3, whereas the same antiserum clearly recognized eIF3j in extracts from HeLa cells (Figure 1B), and from a mouse cell line, N2a (data not shown). Thus, we concluded that our preparation of n-eIF3 lacked eIF3j, which presumably dissociated during purification (Unbehaun *et al*, 2004). As n-eIF3 was able to promote 40S ribosome binding to mRNA (Figure 3), eIF3j is not essential for translation initiation in agreement with other reports (Unbehaun *et al*, 2004; Kolupaeva *et al*, 2005). Rather, eIF3j may serve as a regulator of eIF3 function, since stable binding of eIF3 to the 40S ribosome requires eIF3j (Fraser *et al*, 2004). We did not identify GA-17 (eIF3m), a protein recently discovered in a n-eIF3 sample (Unbehaun *et al*, 2004) that was also identified as a membrane-bound receptor for herpes simplex virus (Perez *et al*, 2005). Hence, eIF3a, eIF3b, eIF3c, eIF3d, eIF3e, eIF3f, eIF3g, eIF3h, eIF3i, eIF3k, and eIF3l should constitute a functional mammalian eIF3 complex.

S. cerevisiae eIF3 comprises only five subunits, eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i (Asano *et al*, 1998; Phan *et al*, 1998). The key question is which subunits of mammalian eIF3 are essential for eIF3 complex formation? In other words, which combination of subunits constitutes the core of the mammalian eIF3? To address this question, we reconstituted the mammalian eIF3 complex by coexpressing human eIF3 subunits. We used a baculovirus-based coexpression system (Fukuda *et al*, 2001) (Figure 2A). Baculovirus-1 system expressed eIF3a-His, eIF3c-FLAG, and eIF3k. His and FLAG-tags were added to eIF3a and eIF3c, respectively, to facilitate purification. Baculovirus-2 expressed eIF3b, eIF3g, eIF3i, and eIF3l, while eIF3d, eIF3e, eIF3f, and eIF3h were expressed from Baculovirus-3 (Figure 2A and B). Insect cells were simultaneously infected with these three recombinant baculoviruses, and the cell extracts were subjected to successive chromatography steps: Ni-NTA agarose resin, gel filtration using Sephacryl S-300, and anti-FLAG resin (Figure 2C). The eluate from the nickel resin, which contained eIF3a-His and its associated proteins, was fractionated by the gel filtration, and each fraction was analyzed by SDS–PAGE and Western blotting for the presence of each subunit (Figure 2D). All of the subunits were present in a high molecular mass (>440 kDa) region (Figure 2D, fractions 2–6). As the n-eIF3 fractionated similarly on the same gel-filtration column (Figure 2D, bottom panel), these fractions were likely to contain the recombinant eIF3 complex and were therefore subjected to anti-FLAG chromatography. SDS–PAGE analysis showed that the recombinant eIF3 complex purified using anti-FLAG resin had a protein composition very similar to that of n-eIF3 (Figure 3A). The presence of all 11 subunits in the recombinant complex was verified by

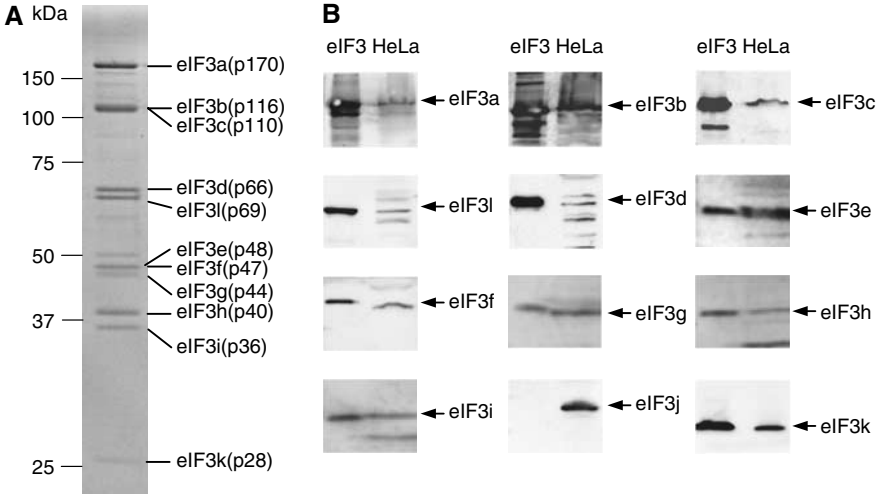

Figure 1 Identification of mammalian eIF3 subunits. (**A**) n-eIF3 (10 μg) was resolved by SDS–PAGE (10%) and stained with CBB. Each band was identified by MALDI-TOF/MS analysis. (**B**) Immunological identification of eIF3 subunits. n-eIF3 (10 μg) and HeLa cell extracts (75 μg protein) were resolved by SDS–PAGE and analyzed by Western blotting using specific antiserum against each subunit.

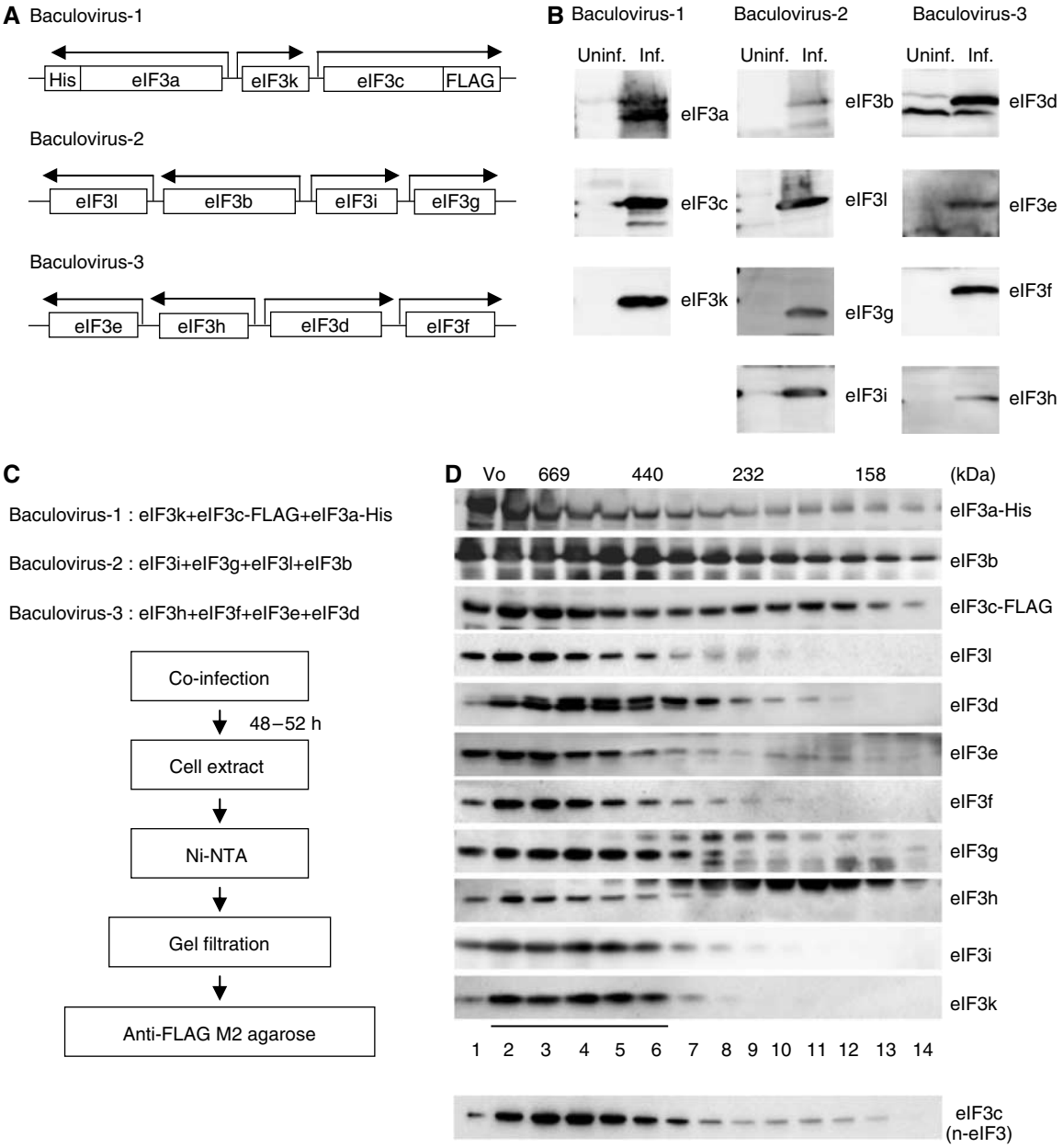

Figure 2 Coexpression system for recombinant eIF3 complex formation. (**A**) Three recombinant baculoviruses were used to express the eIF3 subunits. Arrows indicate the direction of transcription. (**B**) Expression of eIF3 subunits in Sf9 cells. Sf9 cells were infected with baculovirus-1, -2, or -3. Extracts (75 μg protein) from infected cells (inf.) and uninfected cells (uninf.) were resolved by SDS–PAGE and analyzed by Western blotting. (**C**) Diagram of recombinant eIF3 expression and purification protocol. (**D**) Gel filtration of eIF3a-His and associated subunits. Proteins eluted from the Ni-NTA resin were resolved by Sephacryl S-300 as described in Materials and methods. Aliquots of each fraction were analyzed by Western blotting with antiserum against each subunit. Approximate positions of the molecular weight reference proteins (thyroglobulin: 669 kDa; ferritin: 440 kDa; catalase: 232 kDa; aldolase: 158 kDa) on the chromatography are indicated above the panels. The fractions used for further purification are underlined. Bottom panel: n-eIF3 was fractionated in the same manner and analyzed with anti-eIF3c. Vo: void volume.

Western blotting (data not shown). A protein band that was found only in the recombinant eIF3 indicated by the asterisk was identified by MALDI-TOF/MS analysis as a C-terminal portion of eIF3c. This recombinant eIF3 complex consisting of the 11 subunits was subsequently referred to as r-eIF3(11). The yield of r-eIF3(11) was approximately 80 μg from a 3-l insect cell culture.

To determine whether r-eIF3(11) could promote translation initiation, a ribosome binding–toeprinting assay (Morino *et al*, 2000) was performed with purified translation initiation factors and β-globin mRNA (Figure 3B). In the presence of all the required factors including n-eIF3, a 48S ribosomal complex was formed on the initiation AUG as evidenced by the toeprint band (indicated by 'C'), in contrast, in the absence of eIF3, almost no 48S ribosomal complex formed, confirming that 48S ribosomal complex formation is dependent on eIF3. When r-eIF3(11) was used as a source of eIF3, the ribosomal complex was formed at 60–70% the efficiency of the formation with n-eIF3 (Figure 3B). This provided evidence that r-eIF3(11) was functionally active.

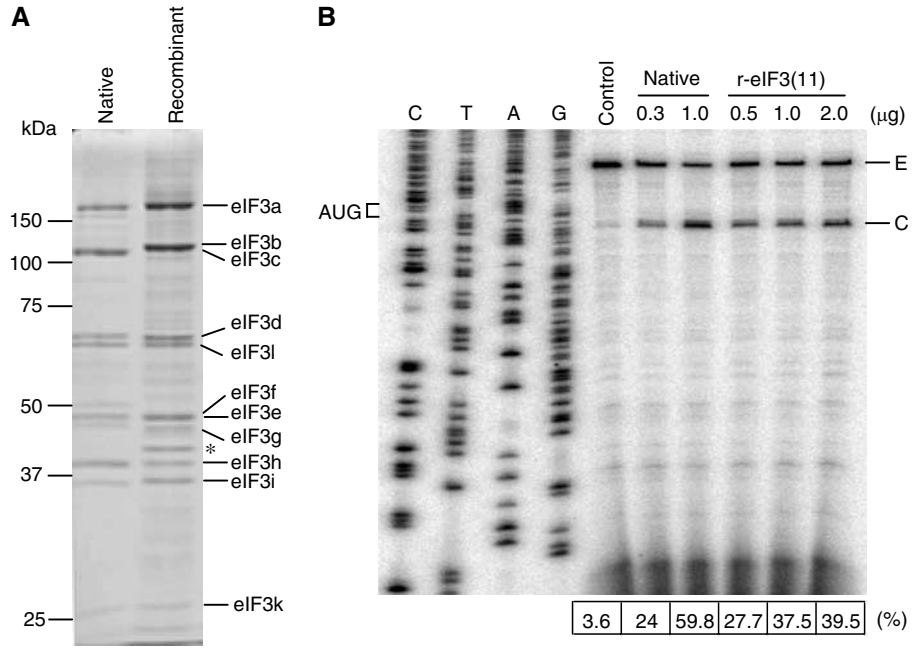

Figure 3 Recombinant eIF3 activity. (**A**) Native-eIF3 (n-eIF3) and recombinant eIF3, r-eIF3(11), (1 μg each) were resolved by SDS–PAGE (10%) and stained by CBB. The asterisk indicates a C-terminal fragment of eIF3c. (**B**) Functional assay of r-eIF3(11). Toeprint analysis of 48S ribosomal complex formation on β-globin mRNA was performed with n-eIF3 or r-eIF3(11). Control: no eIF3. The efficiency (%) of the ribosomal complex formation on the initiator AUG is shown below each lane. C: toeprint complex. E: full-length cDNA.

Dispensable non-conserved subunits

We then examined whether any of the subunits that are not evolutionarily conserved between human and *S. cerevisiae* (i.e. eIF3d, eIF3l, eIF3e, eIF3f, eIF3h, and eIF3k) were dispensable for the formation of an active mammalian eIF3 complex. For this purpose, we expressed 10 eIF3 subunits (the five conserved subunits plus five non-conserved subunits), and attempted to purify an eIF3 complex lacking one of the non-conserved subunits using the same protocol as for r-eIF3(11) isolation. r-eIF3 complexes lacking eIF3d or eIF3l, herein referred to as r-eIF3(del-d) or r-eIF3(del-l), were purified (Figure 4A). The absence of the eIF3l and eIF3d subunits in r-eIF3(del-l) and r-eIF3(del-d), respectively, was verified by Western blotting (Figure 4B). The protein band that was reproducibly present only in r-eIF3(del-d) (indicated by the asterisk in Figure 4A) could not be identified by MALDI-TOF/MS analysis. Both r-eIF3(del-d) and r-eIF3(del-l) promoted the ribosome binding to mRNA and scanning to the AUG codon at ~70% the efficiency with r-eIF3(11) (Figure 4C), indicating that the eIF3d and eIF3l subunits are not essential for the formation of an active mammalian eIF3 complex. It is noteworthy that the r-eIF3(del-l) preparation lacked protein bands corresponding to eIF3g and eIF3i, two evolutionarily conserved subunits (Figure 4A), yet retained the capacity to promote the ribosomal binding to mRNA and scanning to the AUG codon (Figure 4C). This observation suggested that both eIF3g and eIF3i are also dispensable for r-eIF3 function, a finding that was later addressed directly in experiments described below.

In a similar manner, we purified r-eIF3(del-k). Although the absence of eIF3k in r-eIF3(del-k) was confirmed by Western blotting (Figure 4E), CBB-staining of the same protein preparation detected a band at the eIF3k position on SDS–PAGE (Figure 4D). The MALDI-TOF/MS analysis indicated that this contaminant was also present in r-eIF3(11), but it could not be identified. The toeprint analysis showed that the absence of eIF3k had no effect on the eIF3 activity (Figure 4F), indicating that eIF3k is a dispensable subunit.

We were unable to purify r-eIF3(del-e), r-eIF3(del-f), and r-eIF3(del-h), although the expression levels of each subunit in the baculovirus-infected cells were similar to the expression levels when r-eIF3 (11) was purified (data not shown). Taken together, these results suggest that three non-conserved subunits (eIF3d, eIF3l, and eIF3k) are dispensable for the formation of active eIF3 complexes.

Dispensable conserved subunits

Subunits eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i are conserved between human and budding yeast, and these five subunits constitute the yeast eIF3 (Asano *et al*, 1998; Phan *et al*, 1998). To examine whether any of these conserved subunits is dispensable for the formation of the mammalian eIF3 complex, we first tried to reconstitute an eIF3 complex lacking one of the three large subunits, eIF3a, eIF3b, or eIF3c. To reconstitute an eIF3 complex lacking eIF3c, eIF3b-FLAG, eIF3a-His, and the other eight subunits were coexpressed, and a protein complex was purified using the same procedure as for r-eIF3(11) (Figure 5A). The absence of the eIF3c subunit was verified by Western blotting (Figure 5B). As the r-eIF3(del-c) complex exhibited very little activity in the toeprint assay (10% without eIF3c as compared to 40% with it; Figure 5C), eIF3c appears to be important for eIF3 activity. The reduced activity of r-eIF3(del-c) is not due to a relatively smaller amount of eIF3a in the preparation of r-eIF3(del-c) (Figure 5A), but due to the lack of eIF3c, because excess amounts of r-eIF3(del-c) (up to 3.0 μg, as compared with 1.0 μg for the wild type) were used in the functional analysis (Figure 5C). No eIF3 protein complex lacking eIF3a or eIF3b was obtained (data not shown).

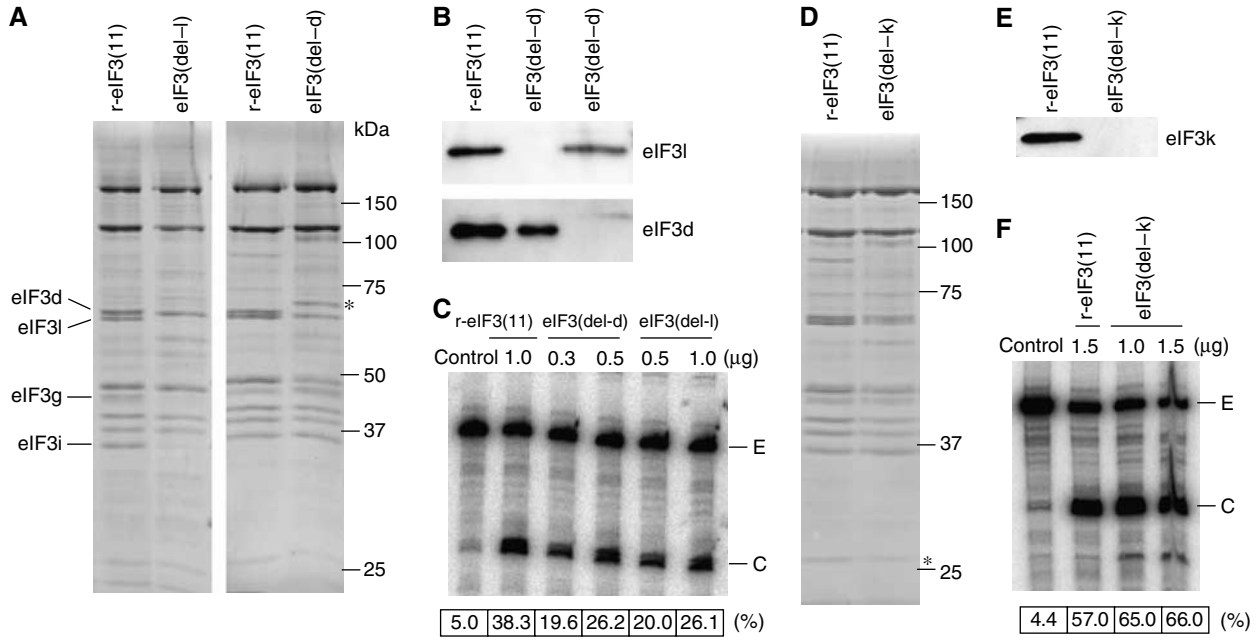

Figure 4 Analysis of recombinant eIF3 lacking eIF3d, eIF3l, or eIF3k subunit. (**A**) r-eIF3(11), r-eIF3(del-d), and r-eIF3(del-l) (0.5 μg each) were resolved by SDS–PAGE and stained by CBB. The asterisk indicates an unidentified contaminant. (**B**) Western blot analysis of r-eIF3(11), r-eIF3(del-d), and r-eIF3(del-l) with anti-eIF3l or anti-eIF3d antiserum. (**C**) Toeprint analysis of r-eIF3(11), r-eIF3(del-d), and r-eIF3(del-l) was performed as in Figure 3B. (**D**) r-eIF3(11) and r-eIF3(del-k) (0.5 μg each) were resolved by SDS–PAGE and stained by CBB. The asterisk indicates the position of eIF3k. (**E**) Western blot analysis of r-eIF3(11) and r-eIF3(del-k) with anti-eIF3k antiserum. (**F**) Toeprint analysis of r-eIF3(11) and r-eIF3(del-k) was performed as in Figure 3B.

We next examined whether the other conserved subunits eIF3g and eIF3i were required for the formation of the active mammalian eIF3 complex. By expressing all the subunits (10 subunits) except eIF3i, r-eIF3(del-i) was successfully purified (Figure 5D). Importantly, eIF3g was also missing in this complex (CBB staining, Figure 5D; Western blot, Figure 5E). In a similar fashion, by expressing all the subunits except eIF3g, r-eIF3(del-g) was purified, and the eIF3i subunit was missing in the complex (Figure 5D and E). Earlier studies reported that eIF3g and eIF3i in budding yeast bind to each other (Verlhac *et al*, 1997; Asano *et al*, 1998). In agreement with these reports, our data support the idea that the evolutionarily conserved subunits eIF3g and eIF3i also interact in the mammalian eIF3 complex. Both r-eIF3(del-g) and r-eIF3(del-i), which lacked both eIF3g and eIF3i, promoted the ribosomal recruitment to mRNA and scanning to the AUG codon at 80–90% the efficiency with r-eIF3(11) (Figure 5F), indicating that the eIF3g and eIF3i subunits are not essential for the formation of active mammalian eIF3 complex.

To verify these results, we silenced the expression of eIF3g and eIF3i in HeLa cells using RNAi. Transfection with siRNA against eIF3g mRNA decreased eIF3g expression by 80% (Figure 6A, middle panel). Interestingly, eIF3i expression was also reduced by 60% (Figure 6A, lower panel). This result corroborates an earlier observation that eIF3g depletion in budding yeast was accompanied by a strong reduction in eIF3i (Hanachi *et al*, 1999), and suggests that eIF3g is required for stabilization of eIF3i in eukaryotic cells. Transfection with siRNA against eIF3i mRNA did not effectively decrease the protein level of eIF3i. To examine whether the concomitant reduction in protein levels of eIF3g and eIF3i influenced the rate of protein synthesis, HeLa cells transfected with siRNA against eIF3g or control siRNA were metabolically labeled with ^{35}S-methionine, and the radioactivity incorporated into proteins was quantified. The rate of protein synthesis did not change significantly by eIF3g knockdown (Figure 6B). When each of three different siRNAs against eIF3c was used for transfection, about 50% of HeLa cells as compared with the cells transfected with the control siRNA were detached from the culture dish in 2 days, whereas transfection of siRNA against eIF3g did not affect cell growth. The protein level of eIF3c in the cells that were transfected with the eIF3c-siRNA and were still attaching to the dish was unchanged as compared with that in the control siRNA-transfected cells (we normalized the assay for the amount of proteins), and the rate of protein synthesis in these cells was also unchanged. We suspect that HeLa cells depleted of eIF3c were detached from the dish as dying cells, while the cells that were still attaching to the dish were those with a normal level of eIF3c and therefore with a normal translation rate.

To examine whether eIF3 from HeLa cells transfected with the eIF3g-siRNA or control siRNA was indeed depleted of eIF3g and eIF3i, ribosomal salt-washed samples from transfected cells were resolved by sucrose density-gradient centrifugation and analyzed for the presence of eIF3g, eIF3i, eIF3c, and eIF3f (Figure 6C). Whereas eIF3c and eIF3f were detected in the middle fractions (fractions 15–18) of both gradients at similar levels, neither eIF3g nor eIF3i were detected in these eIF3-containing fractions from the eIF3g-siRNA-treated cells (Figure 6C). These results support the idea that eIF3g and eIF3i subunits are not essential for the formation of the mammalian eIF3 complex.

The core of eIF3

Our ultimate goal was to reconstitute the functional core of human eIF3 in order to identify the minimum components

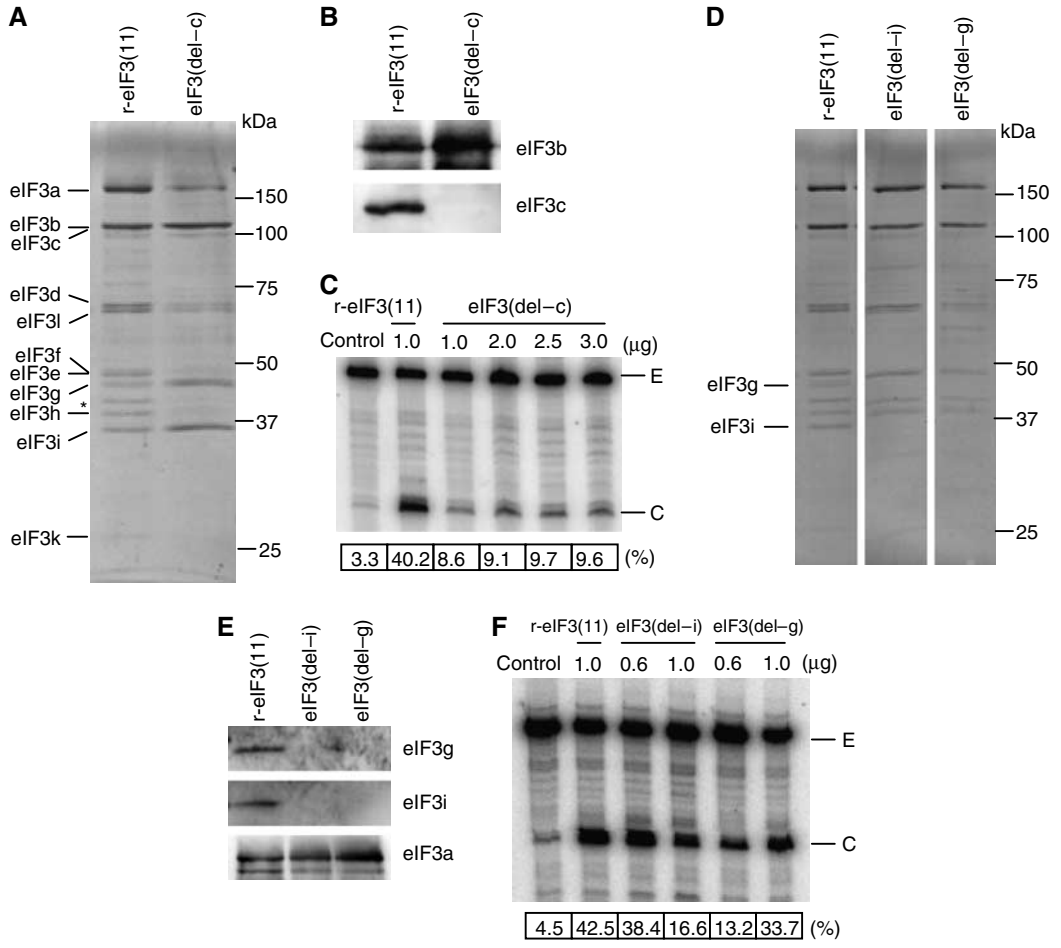

Figure 5 Analysis of recombinant eIF3 lacking eIF3c, eIF3i, or eIF3g subunit. (**A**) r-eIF3(11) and r-eIF3(del-c) (1 μg each) were resolved by SDS–PAGE and stained by CBB. The asterisk indicates a C-terminal fragment of eIF3c. (**B**) Western blotting analysis of r-eIF3(11) and r-eIF3(del-c) with anti-eIF3b or anti-eIF3c antiserum. (**C**) Toeprint analysis of r-eIF3(11) and r-eIF3(del-c) was performed as in Figure 3B. (**D**) r-eIF3(11), r-eIF3(del-i), and r-eIF3(del-g) (1 μg each) were resolved by SDS–PAGE and stained by CBB. (**E**) Western blotting analysis of r-eIF3(11), r-eIF3(del-g), and r-eIF3(del-i) with anti-eIF3g, anti-eIF3i, or anti-eIF3a antiserum. (**F**) Toeprint analysis of r-eIF3(11), r-eIF3(del-i), and r-eIF3(del-g) was performed as in Figure 3B.

required for activity. The data presented above indicated that five subunits: eIF3d, eIF3l, eIF3k, eIF3g, and eIF3i are not required for the formation of active eIF3. We hypothesized that the other six subunits: eIF3a, eIF3b, eIF3c, eIF3e, eIF3f, and eIF3h constituted the core of mammalian eIF3. We therefore expressed these six subunits and purified a complex (Figure 7A). Although we could not obtain this complex in sufficient quantity to detect the eIF3e, eIF3f, and eIF3h subunits clearly by CBB staining, we confirmed the presence of all six subunits by Western blotting (Figure 7A). A complex comprising eIF3a, eIF3b, eIF3e, eIF3f, and eIF3h, but lacking eIF3c was also prepared as negative control (Figure 7A). The six-subunit complex promoted ribosome binding to mRNA, whereas the five-subunit complex did not (Figure 7B). These results suggest that the functional core of mammalian eIF3 comprises eIF3a, eIF3b, eIF3c, eIF3e, eIF3f, and eIF3h.

Discussion

We have successfully reconstituted a functional human eIF3 by coexpressing 11 subunits. This recombinant eIF3 was functional in a 40S ribosome-binding assay using β-globin mRNA. By reconstituting recombinant eIF3 complexes that lacked different individual subunits, we identified the subunits that are dispensable for assembly of an active eIF3 complex. It was striking that two evolutionarily conserved subunits, eIF3g and eIF3i, were not essential for eIF3 activity.

Our findings are in agreement with the structural model of eIF3 proposed for budding yeast in which eIF3g and eIF3i bind to a C-terminal part of eIF3b, but do not play a critical role in the basal activity of eIF3 (Valasek *et al*, 2001, 2002). These two subunits seem to work in concert. When the eIF3g subunit is deleted, the eIF3i subunit is also absent from the recombinant eIF3 complex, and vice versa (Figure 5D and E). Knockdown of the eIF3g subunit in HeLa cells is accompanied by a reduction in the level of the eIF3i subunit (Figure 6); similarly, a reduction in eIF3g expression leads to depletion of eIF3i in yeast (Hanachi *et al*, 1999). The physical association of eIF3g and eIF3i has also been observed in budding yeast (Verlhac *et al*, 1997; Asano *et al*, 1998).

What, then, is the function of the eIF3g and eIF3i subunits? We demonstrated that binding of the 40S ribosome-initiation factor complex at the AUG codon of mRNA does not require the presence of eIF3g and eIF3i (Figure 5F). Furthermore, the rate of overall protein synthesis does not change by knockdown of the eIF3g subunit in HeLa cells (Figure 6B). Thus,

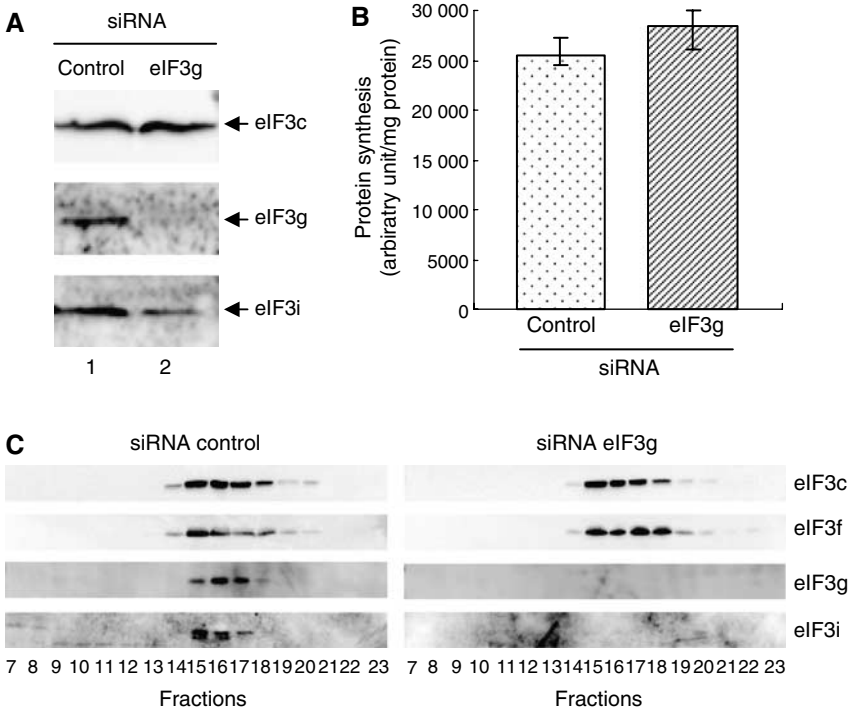

Figure 6 Depletion of eIF3g and eIF3i from n-eIF3 in HeLa cells. (**A**) HeLa cells were transfected with siRNA against eIF3g (lane 2) or control siRNA (lane 1). Cell lysate was analyzed by Western blotting with anti-eIF3c, anti-eIF3g, or anti-eIF3i antiserum. (**B**) HeLa cells transfected with siRNA as in (A) were pulse-labeled with [^{35}S] methionine for 30 min. Cell extracts were resolved by SDS–PAGE, and the incorporated radioactivity of the protein bands was quantified in a combined manner. Data are the mean values of three experiments ± s.d. (**C**) Purification of n-eIF3 depleted of eIF3g and eIF3i. HeLa cells were transfected with siRNA against eIF3g or control siRNA. Ribosomal salt-washed fractions from the transfected cells were resolved by sucrose-density gradient centrifugation. Fractions 7–23 (the bottom of the gradient) were analyzed by Western blotting for eIF3c, eIF3f, eIF3g, and eIF3i.

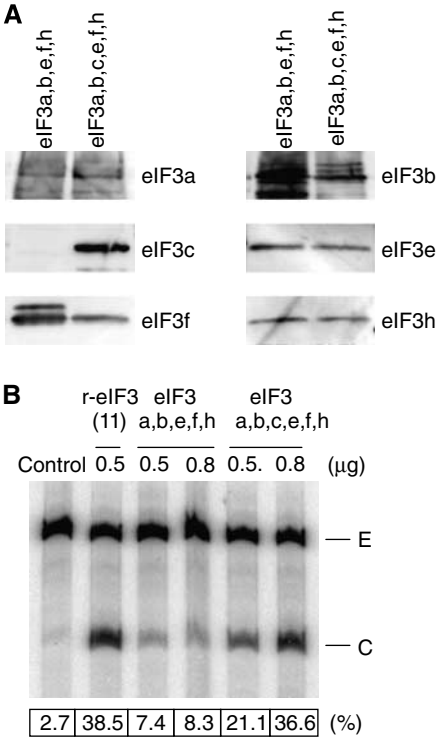

Figure 7 The core of human eIF3. (**A**) Complexes comprising five subunits (eIF3a, eIF3b, eIF3e, eIF3f, and eIF3h) or six subunits (eIF3a, eIF3b, eIF3c, eIF3e, eIF3f, and eIF3h) were resolved by SDS–PAGE and analyzed by Western blotting with relevant antisera. (**B**) Toeprint analysis of the five-or six-subunit complex was performed as in Figure 3B.

these two subunits might be involved in translational control of specific mRNAs or in particular cellular conditions.

We also found that three evolutionarily non-conserved subunits, eIF3d, eIF3l, and eIF3k, are dispensable for the assembly of active human eIF3. We repeatedly attempted to reconstitute r-eIF3 lacking any one of the other non-conserved subunits (eIF3e, eIF3f, and eIF3h), but obtained only small amounts of complexes that were insufficient for assaying the activity (data not shown). These results imply that eIF3e, eIF3f, and eIF3h are important for efficient formation of a functional eIF3 complex. We also demonstrated the importance of eIF3c for eIF3 activity by showing that r-eIF3(del-c) was not very active in the toeprint analysis (Figure 5C), and that transfection of HeLa cells with an siRNA against eIF3c resulted in severely decreased cell viability (data not shown). eIF3c may coordinate the functions of eIF1 and eIF5 by binding these two initiation factors and the 40S ribosome (Valasek *et al*, 2003, 2004). The use of r-eIF3(del-c) in various assay systems such as a completely reconstituted translation system (Alkalaeva *et al*, 2006) with or without eIF1 or eIF5 may provide insight into the role of eIF3c in translation.

In pioneering work using a budding yeast system, Hinnebusch's group demonstrated that a subcomplex consisting of eIF3a, eIF3b, and eIF3c could carry out most eIF3 functions. In this system, the eIF3a subunit was thought to bridge the eIF3b and eIF3c subunits (Phan *et al*, 2001). Physical interaction of eIF3a and eIF3b has been clearly shown both for budding yeast (Asano *et al*, 1998; Valasek *et al*, 2001) and mammals (Methot *et al*, 1997). Although

binding of eIF3c to eIF3a was also evident in budding yeast (Asano *et al*, 1998; Phan *et al*, 2001), such an interaction has not been previously shown for mammals. Fraser *et al* (2004) expressed five mammalian homologues of budding yeast eIF3 (eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i subunits where the eIF3b subunit was FLAG-tagged) in insect cells, and purified a protein complex by anti-FLAG chromatography. The purified complex consisted of eIF3a, eIF3b, eIF3g, and eIF3i, but not eIF3c, suggesting that evolutionarily non-conserved subunits might be required to stably incorporate eIF3c into the four-subunit complex. In the present study, we coexpressed six non-conserved and five conserved eIF3 subunits, and successfully purified an active eIF3 complex. The purified complex contained eIF3c, as eIF3c was FLAG-tagged and the last step of the purification strategy was anti-FLAG-chromatography. The eIF3c molecule was contained in the purified eIF3 complex such that the association of eIF3c with the eIF3 complex was resistant to high ionic conditions during the Ni-NTA and gel-filtration chromatography purification steps. If the important functions of eIF3 are carried out by the largest three subunits, as suggested by the yeast study (Phan *et al*, 2001), the role of eIF3e, eIF3f, and eIF3h might be to tightly bind eIF3c to an eIF3a-eIF3b-associated complex rather than to directly participate in translation initiation. In support of this, when only the evolutionarily conserved subunits (eIF3a, eIF3b, eIF3c-FLAG, eIF3g, and eIF3i) were expressed, far less amounts of eIF3a and eIF3b were copurified with eIF3c-FLAG even under physiological ionic conditions than when all the subunits were expressed (Supplementary Figure S1). Of note, the eIF3e subunit was shown to bind to the eIF3c subunit in mammals (Morris-Desbois *et al*, 1999) and in *Arabidopsis* (Yahalom *et al*, 2001). eIF3e and eIF3c each possess a PCI (proteosome-COP9-initiation factor) domain, and eIF3f and eIF3h have a MPN (Mpr1-Pad1-N-terminal) domain. The PCI and MPN domains mediate protein–protein interactions and are also found in some subunits of the 26S proteasome and in the COP9 signalosome (Chang and Schwechheimer, 2004).

Are eIF3e, eIF3f, and eIF3h subunits essential *in vivo*? In fission yeast, deletion of the eIF3e subunit is not lethal and leads to only a moderate decrease in protein synthesis (Bandyopadhyay *et al*, 2000; Akiyoshi *et al*, 2001). However, the presence of the eIF3e was required for formation of a stable eIF3 complex (Bandyopadhyay *et al*, 2002). In contrast, in fission yeast, eIF3f is essential for growth (Zhou *et al*, 2005). In *Arabidopsis*, disruption of eIF3h is not lethal, and has no effect on global protein synthesis (Kim *et al*, 2004). However, gel filtration of protein extracts from the eIF3h subunit-disrupted plants showed that eIF3 as detected by the presence of eIF3b, eluted in lower molecular weight fractions than did eIF3 from control plants (Kim *et al*, 2004). Thus, as noted above, the eIF3h subunit could affect the integrity of the eIF3 complex. Presumably, the eIF3e, eIF3f, and eIF3h subunits serve to stabilize the eIF3 complex. These subunits may also be involved in translational regulation. Mammalian eIF3e and eIF3f are proposed to bind to eIF4G (LeFebvre *et al*, 2006) and mTOR (Harris *et al*, 2006), respectively. In human cells, the eIF3e subunit mediates translational repression by p56, an interferon-inducible protein (Guo *et al*, 2000). Plant eIF3h is implicated in translation of specific mRNAs (Kim *et al*, 2004).

What are the functions of the eIF3d, eIF3l, and eIF3k subunits? These subunits were dispensable for the formation of active mammalian eIF3 in the present study. However, deletion of eIF3d, which did not result in lethality, rendered the eIF3 complex physically unstable in fission yeast (Bandyopadhyay *et al*, 2002). eIF3d binds to eIF3e in fission yeast (Yen and Chang, 2000; Bandyopadhyay *et al*, 2002), while interactions of mammalian eIF3l with eIF3e (Morris-Desbois *et al*, 2001) and of mammalian eIF3k with the eIF3c, eIF3g, and eIF3j subunits (Mayeur *et al*, 2003) have been reported. These intersubunit connections probably increase the physical stability of eIF3.

A pertinent question about the coexpression system used in this study is whether endogenous eIF3 subunits derived from insect cells were incorporated into the recombinant mammalian eIF3 complex. It is unlikely that significant incorporation took place due to the much greater abundance of the recombinant subunits: the recombinant subunits in cell extracts expressed by the baculovirus system are detectable by CBB staining on SDS–PAGE, while those of insect eIF3 are apparently undetectable (data not shown).

Finally, in the present study, we assayed r-eIF3 in toeprint analysis using β-globin mRNA. However, β-globin mRNA is translated in a cap-dependent manner. It will be important to examine whether different combinations of eIF3 subunits would suffice for translation initiation on IRES-containing mRNA such as EMCV (Pestova *et al*, 1996) or HCV (Pestova *et al*, 1998). The toeprint analysis in the present study monitors only the formation of the 48S ribosomal complex, but not of the 80S complex, leaving the possibility that different subunits of eIF3 are required at different stages of translation initiation. In addition, eIF3 probably participates not only in initiation but also in termination followed by reinitiation of translation as well (Nielsen *et al*, 2004; Kolupaeva *et al*, 2005). Thus, it is important to test recombinant eIF3 deletion mutants in a completely reconstituted translation system (Alkalaeva *et al*, 2006) to examine whether different subunits take part in different phases of translation.

Materials and methods

cDNAs and plasmids

The cDNA for eIF3b was described previously (Methot *et al*, 1997). cDNAs for eIF3c (Asano *et al*, 1997a), eIF3d (Asano *et al*, 1997c), eIF3e (Asano *et al*, 1997b), eIF3f (Asano *et al*, 1997c), eIF3g (Block *et al*, 1998), eIF3h (Asano *et al*, 1997c), eIF3i (Asano *et al*, 1997a), and eIF3j (Block *et al*, 1998) were kind gifts from Dr JWB Hershey (University of California, Davis). cDNAs for eIF3a, eIF3l, and eIF3k were obtained by reverse transcription followed by the polymerase chain reaction using RNA from HeLa cells and DNA primers designed based on reported sequences (GenBank accession numbers: U78311 for eIF3a, AF077207 for eIF3l, AF085358 for eIF3k).

His-tag and FLAG-tag sequences were added to the C termini of the eIF3a and eIF3c coding regions, respectively, to facilitate protein purification. The multicloning sites of pAcDB3 and pAcAB4 (BD Biosciences) were modified to harbor additional restriction enzyme sites (*Xho*I, *Eco*RV, *Kpn*I, *Sal*I, *Hpa*I, *Not*I, *Nhe*I, and *Nde*I) and (*Eco*RV, *Not*I, *Pst*I, *Nhe*I, and *Sac*I) to generate pAcDB3(m) and pAcAB4(m), respectively. eIF3a-His, eIF3c-FLAG, and eIF3k cDNAs were cloned in combination in pAcDB3(m) to produce pAcDB3-eIF3a-His/eIF3c-FLAG/eIF3k. eIF3b, eIF3l, eIF3g, and eIF3i cDNAs were cloned together pAcAB4(m) to generate pAcAB4-eIF3b/eIF3l/eIF3g/eIF3i. eIF3d, eIF3e, eIF3f, and eIF3h cDNAs were incorporated into pAcAB4(m) to produce pAcAB4-eIF3d/eIF3e/eIF3f/eIF3h. The construction of plasmids for deletion mutants of r-eIF3 is described in Supplementary data.

Baculoviruses

For r-eIF3(11), baculovirus-1, which expresses eIF3a-His, eIF3c-FLAG and eIF3k, baculovirus-2, which expresses eIF3b, eIF3l, eIF3g and eIF3i, and baculovirus-3, which expresses eIF3d, eIF3e, eIF3f, and eIF3h were generated in Sf9 cells by co-transfection of baculovirus DNA BaculoGold (BD Biosciences) with pAcDB3-eIF3a-His/eIF3c-FLAG/eIF3k, pAcAB4-eIF3b/eIF3l/eIF3g/eIF3i, and pAcAB4-eIF3d/eIF3e/eIF3f/eIF3h, respectively. To generate baculoviruses for expressing deletion mutants of r-eIF3, relevant plasmids were used for transfection. Recombinant baculoviruses were purified from isolated plaques using a standard procedure and amplified by infection of Sf9 cells ($1.5–2.5 \times 10^6$ cells/ml, 300–600 ml) followed by culturing until more than 90% of cells were disrupted. After removing cell debris by centrifugation (800 *g* for 15 min), the supernatant was kept at 4°C in the dark.

Expression and purification of recombinant eIF3

High-Five cells (Invitrogen; 3 l) were grown by Cell-master controller (Wakenyaku, Japan; O_2 concentration, 7.9 p.p.m.; temperature, 27°C) and infected at $0.8–1.1 \times 10^6$ cells/ml with a baculovirus stock expressing eIF3a (600 ml) and two other baculovirus stocks (200 ml each) simultaneously. After 48–52 h, cells were harvested and suspended in 300 ml buffer (0.5 M KCl, 20 mM HEPES-KOH, pH 7.5, 10% glycerol, 5 mM 2-mercaptoethanol, 0.5% Triton X-100, and a protease inhibitor cocktail: complete EDTA-free (Roche)) and incubated on ice for 20 min. After centrifugation at 9000 r.p.m. for 30 min, the supernatant was supplemented with imidazole (20 mM) and mixed with Ni-NTA agarose resin (Qiagen; 16 ml). Following incubation at 4°C for 1 h with constant rotation, the mixture was centrifuged at 3000 r.p.m. for 3 min, and the resin was washed twice with buffer (150 ml; 0.5 M KCl, 20 mM HEPES-KOH, pH 7.5, 10% glycerol, 5 mM of 2-mercaptoethanol, 0.01% Triton X-100, 20 mM imidazole). Proteins bound to the resin were eluted out with imidazole buffer (56 ml; 250 mM imidazole, 0.67 M KCl, 20 mM HEPES-KOH, pH 7.5, 10% glycerol, 5 mM of 2- mercaptoethanol, complete EDTA-free). The eluate was concentrated to 5 ml using AMICON Ultra MWCO 10000 (Millipore), and then applied to Sephacryl S-300 (16/60) (Amersham). Proteins were eluted (0.3 ml/min, 1 ml/fraction) with buffer (0.5 M KCl, 20 mM HEPES-KOH, pH 7.5, 10% glycerol, 5 mM of 2-mercaptoethanol, 1 mM EDTA), and an aliquot of each fraction was analyzed for the presence of expressed eIF3 subunits by Western blotting. Fractions containing relevant subunits were diluted with 3 × volume buffer (20 mM HEPES-KOH pH 7.5, 10% glycerol, 5 mM of 2-mercaptoethanol, and Roche complete protease inhibitor cocktail), and applied to anti-FLAG agarose column (Sigma; 0.2 ml). After washing with buffer (10 ml; 0.1 M KCl, 20 mM HEPES-KOH, pH 7.5, 10% glycerol, 5 mM of 2-mercaptoethanol, and Roche complete protease inhibitor cocktail), proteins were eluted with buffer (600 µl; 0.1 M KCl, 20 mM HEPES-KOH pH 7.5, 25% glycerol, 5 mM of 2-mercaptoethanol, 0.1 mM EDTA) containing FLAG peptide (Sigma; 100 µg/ml).

n-eIF3 was purified from Krebs-2 ascites cells as described (Trachsel *et al*, 1979; Mikami *et al*, 2006b).

Toeprint analysis

All the eIFs were purified as described (Mikami *et al*, 2006a, b). Toeprint analysis was performed essentially as described (Morino *et al*, 2000) with the following modifications. eIF2 (1 µg) was preincubated with GTP (3 mM) and Met-tRNAi^{Met} (0.7 pmol) at 26°C for 20 min to enhance the ternary complex (eIF2-GTP-Met-tRNAi^{Met}) formation. The ternary complex was mixed with His-eIF1 (0.5 µg), His-eIF1A (0.5 µg), eIF4E (0.3 µg), FLAG-eIF4G-His (0.9 µg), eIF4A (2 µg), FLAG-eIF4B (0.5 µg), 40S ribosome (1.4 pmol), and n-eIF3 (0.3–1 µg) or recombinant eIF3 (0.3–2.5 µg). β-globin mRNA (50 ng; Gibco BRL) was heated at 70°C with a ^{32}P-labeled primer (5′-GCATTTGCAGAGGACAGG; 2 pmol) for 2 min and then gradually cooled to 60°C and chilled on ice. The mixture of eIFs, 40S ribosome, and the mRNA-primer complex was incubated at 30°C for 5 min in buffer (50 µl; 100 mM KCl, 3 mM magnesium acetate, 13 mM HEPES-KOH, pH 7.5, 0.25 mM spermidine, 1 mM DTT, 0.6 mM GTP, 1.8 mM ATP). A reverse transcriptase mixture (50 µl; 40 mM KCl, 8 mM magnesium acetate, 80 mM Tris–HCl, pH 7.5, 10 mM DTT, 1 mM dGTP, 1 mM dATP, 1 mM dCTP, 1 mM dTTP) containing SuperScript II (100 units; Invitrogen) was then added and incubated at 37°C for 10 min. After the reverse transcription reaction, RNase A (10 µg) was added and incubated at 37°C for 5 min, followed by treatment with phenol/chloroform and precipitation with ethanol. The same primer was used for sequencing of a plasmid harboring β-globin cDNA. The products of primer extension and sequencing were resolved on PAGE (8%) containing 6 M urea and visualized using a BAS-2000 phosphor-imager (Fuji). In the figures, the full-length cDNA and toeprint products are marked 'E' for 'end-product' and 'C' for 'complex', respectively. The intensity of 'E' and 'C' was quantified by BAS-2000. The efficiency of the ribosomal complex formation on the initiator AUG was calculated as C/(E + C). We repeated each toeprint analysis at least twice and obtained reproducible results.

Mass spectrometric analysis, antibodies and siRNA experiments are described in Supplementary data.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

Acknowledgements

We thank Dr JMB Hershey for eIF3 subunit cDNAs, Dr K Hisatake for plasmid construction advice, and Dr S Morino for initiating this project. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan to HI, by the RIKEN Structural Genomics/Proteomics Initiative (RSGI), and by the National Project on Protein Structural and Functional Analyses (Ministry of Education, Culture, Sports, Science and Technology of Japan).

References

Akiyoshi Y, Clayton J, Phan L, Yamamoto M, Hinnebusch AG, Watanabe Y, Asano K (2001) Fission yeast homolog of murine Int-6 protein, encoded by mouse mammary tumor virus integration site, is associated with the conserved core subunits of eukaryotic translation initiation factor 3. *J Biol Chem* **276:** 10056–10062

Alkalaeva EZ, Pisarev AV, Frolova LY, Kisselev LL, Pestova TV (2006) *In vitro* reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell* **125:** 1125–1136

Asano K, Kinzy TG, Merrick WC, Hershey JW (1997a) Conservation and diversity of eukaryotic translation initiation factor eIF3. *J Biol Chem* **272:** 1101–1109

Asano K, Merrick WC, Hershey JW (1997b) The translation initiation factor eIF3-p48 subunit is encoded by int-6, a site of frequent integration by the mouse mammary tumor virus genome. *J Biol Chem* **272:** 23477–23480

Asano K, Phan L, Anderson J, Hinnebusch AG (1998) Complex formation by all five homologues of mammalian translation initiation factor 3 subunits from yeast *Saccharomyces cerevisiae*. *J Biol Chem* **273:** 18573–18585

Asano K, Vornlocher HP, Richter-Cook NJ, Merrick WC, Hinnebusch AG, Hershey JW (1997c) Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits. Possible roles in RNA binding and macromolecular assembly. *J Biol Chem* **272:** 27042–27052

Bandyopadhyay A, Lakshmanan V, Matsumoto T, Chang EC, Maitra U (2002) Moe1 and spInt6, the fission yeast homologues of mammalian translation initiation factor 3 subunits p66 (eIF3d) and p48 (eIF3e), respectively, are required for stable association of eIF3 subunits. *J Biol Chem* **277:** 2360–2367

Bandyopadhyay A, Matsumoto T, Maitra U (2000) Fission yeast Int6 is not essential for global translation initiation, but deletion of int6(+) causes hypersensitivity to caffeine and affects spore formation. *Mol Biol Cell* **11:** 4005–4018

Baugh JM, Pilipenko EV (2004) 20S proteasome differentially alters translation of different mRNAs via the cleavage of eIF4F and eIF3. *Mol Cell* **16:** 575–586

Behlke J, Bommer UA, Lutsch G, Henske A, Bielka H (1986) Structure of initiation factor eIF-3 from rat liver. Hydrodynamic and electron microscopic investigations. *Eur J Biochem* **157:** 523–530

Block KL, Vornlocher HP, Hershey JW (1998) Characterization of cDNAs encoding the p44 and p35 subunits of human translation initiation factor eIF3. *J Biol Chem* **273:** 31901–31908

Chang EC, Schwechheimer C (2004) ZOMES III: the interface between signalling and proteolysis. Meeting on the COP9 signalosome, proteasome and eIF3. *EMBO Rep* **5:** 1041–1045

Chaudhuri J, Chowdhury D, Maitra U (1999) Distinct functions of eukaryotic translation initiation factors eIF1A and eIF3 in the formation of the 40 S ribosomal preinitiation complex. *J Biol Chem* **274:** 17975–17980

Danaie P, Wittmer B, Altmann M, Trachsel H (1995) Isolation of a protein complex containing translation initiation factor Prt1 from *Saccharomyces cerevisiae*. *J Biol Chem* **270:** 4288–4292

Fraser CS, Lee JY, Mayeur GL, Bushell M, Doudna JA, Hershey JW (2004) The j-subunit of human translation initiation factor eIF3 is required for the stable binding of eIF3 and its subcomplexes to 40 S ribosomal subunits in vitro. *J Biol Chem* **279:** 8946–8956

Fukuda A, Yamauchi J, Wu SY, Chiang CM, Muramatsu M, Hisatake K (2001) Reconstitution of recombinant TFIIH that can mediate activator-dependent transcription. *Genes Cells* **6:** 707–719

Gingras AC, Raught B, Sonenberg N (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* **68:** 913–963

Greenberg JR, Phan L, Gu Z, deSilva A, Apolito C, Sherman F, Hinnebusch AG, Goldfarb DS (1998) Nip1p associates with 40 S ribosomes and the Prt1p subunit of eukaryotic initiation factor 3 and is required for efficient translation initiation. *J Biol Chem* **273:** 23485–23494

Guo J, Hui DJ, Merrick WC, Sen GC (2000) A new pathway of translational regulation mediated by eukaryotic initiation factor 3. *EMBO J* **19:** 6891–6899

Hanachi P, Hershey JW, Vornlocher HP (1999) Characterization of the p33 subunit of eukaryotic translation initiation factor-3 from *Saccharomyces cerevisiae*. *J Biol Chem* **274:** 8546–8553

Harris TE, Chi A, Shabanowitz J, Hunt DF, Rhoads RE, Lawrence Jr JC (2006) mTOR-dependent stimulation of the association of eIF4G and eIF3 by insulin. *EMBO J* **25:** 1659–1668

Hershey JW, Asano K, Naranda T, Vornlocher HP, Hanachi P, Merrick WC (1996) Conservation and diversity in the structure of translation initiation factor EIF3 from humans and yeast. *Biochimie* **78:** 903–907

Hershey JWB, Merrick WC (2000) The pathway and mechanism of initiation of protein synthesis. In *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds), pp 33–88. Cold Spring Harbor: Cold Spring Harbor Laboratory

Hinnebusch A (2000) Mechanism and regulation of initiator methionyl-tRNA binding to ribosomes. In *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds), pp 185–243. Cold Spring Harbor: Cold Spring Harbor Laboratory

Hinnebusch AG (2006) eIF3: a versatile scaffold for translation initiation complexes. *Trends Biochem Sci* **31:** 553–562

Holz MK, Ballif BA, Gygi SP, Blenis J (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* **123:** 569–580

Kim TH, Kim BH, Yahalom A, Chamovitz DA, 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

Kolupaeva VG, Unbehaun A, Lomakin IB, Hellen CU, Pestova TV (2005) Binding of eukaryotic initiation factor 3 to ribosomal 40S subunits and its role in ribosomal dissociation and anti-association. *RNA* **11:** 470–486

LeFebvre AK, Korneeva NL, Trutschl M, Cvek U, Duzan RD, Bradley CA, Hershey JW, Rhoads RE (2006) Translation initiation factor eIF4G-1 binds to eIF3 through the eIF3e subunit. *J Biol Chem* **281:** 22917–22932

Mayeur GL, Fraser CS, Peiretti F, Block KL, Hershey JW (2003) Characterization of eIF3k: a newly discovered subunit of mammalian translation initiation factor eIF3. *Eur J Biochem* **270:** 4133–4139

Mengod G, Trachsel H (1985) Eukaryotic protein synthesis initiation factor eIF-3: determination of concentration and association with ribosomes in rabbit reticulocyte and HeLa cell lysates. *Biochim Biophys Acta* **825:** 169–174

Methot N, Rom E, Olsen H, Sonenberg N (1997) The human homologue of the yeast Prt1 protein is an integral part of the eukaryotic initiation factor 3 complex and interacts with p170. *J Biol Chem* **272:** 1110–1116

Meyer LJ, Milburn SC, Hershey JW (1982) Immunochemical characterization of mammalian protein synthesis initiation factors. *Biochemistry* **21:** 4206–4212

Mikami S, Kobayashi T, Yokoyama S, Imataka H (2006a) A hybridoma-based *in vitro* translation system that efficiently synthesizes glycoproteins. *J Biotechnol* **127:** 65–78

Mikami S, Masutani M, Sonenberg N, Yokoyama S, Imataka H (2006b) An efficient mammalian cell-free translation system supplemented with translation factors. *Protein Expr Purif* **46:** 348–357

Miyamoto S, Patel P, Hershey JW (2005) Changes in ribosomal binding activity of eIF3 correlate with increased translation rates during activation of T lymphocytes. *J Biol Chem* **280:** 28251–28264

Morino S, Imataka H, Svitkin YV, Pestova TV, Sonenberg N (2000) Eukaryotic translation initiation factor 4E (eIF4E) binding site and the middle one-third of eIF4GI constitute the core domain for cap-dependent translation, and the C-terminal one-third functions as a modulatory region. *Mol Cell Biol* **20:** 468–477

Morris-Desbois C, Bochard V, Reynaud C, Jalinot P (1999) Interaction between the Ret finger protein and the Int-6 gene product and co-localisation into nuclear bodies. *J Cell Sci* **112** (Part 19)**:** 3331–3342

Morris-Desbois C, Rety S, Ferro M, Garin J, Jalinot P (2001) The human protein HSPC021 interacts with Int-6 and is associated with eukaryotic translation initiation factor 3. *J Biol Chem* **276:** 45988–45995

Naranda T, Kainuma M, MacMillan SE, Hershey JW (1997) The 39-kilodalton subunit of eukaryotic translation initiation factor 3 is essential for the complex's integrity and for cell viability in *Saccharomyces cerevisiae*. *Mol Cell Biol* **17:** 145–153

Nielsen KH, Szamecz B, Valasek L, Jivotovskaya A, Shin BS, Hinnebusch AG (2004) Functions of eIF3 downstream of 48S assembly impact AUG recognition and GCN4 translational control. *EMBO J* **23:** 1166–1177

Perez A, Li QX, Perez-Romero P, Delassus G, Lopez SR, Sutter S, McLaren N, Fuller AO (2005) A new class of receptor for herpes simplex virus has heptad repeat motifs that are common to membrane fusion proteins. *J Virol* **79:** 7419–7430

Pestova TV, Hellen CU, Shatsky IN (1996) Canonical eukaryotic initiation factors determine initiation of translation by internal ribosomal entry. *Mol Cell Biol* **16:** 6859–6869

Pestova TV, Shatsky IN, Fletcher SP, Jackson RJ, Hellen CU (1998) A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev* **12:** 67–83

Phan L, Schoenfeld LW, Valasek L, Nielsen KH, Hinnebusch AG (2001) A subcomplex of three eIF3 subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and tRNA(i)Met. *EMBO J* **20:** 2954–2965

Phan L, Zhang X, Asano K, Anderson J, Vornlocher HP, Greenberg JR, Qin J, Hinnebusch AG (1998) Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5. *Mol Cell Biol* **18:** 4935–4946

Raught B, Gingeras AC, Sonenberg N (2000) Regulation of ribosomal recruitment in eukaryotes. In *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds), pp 245–293. Cold Spring Harbor: Cold Spring Harbor Laboratory

Siridechadilok B, Fraser CS, Hall RJ, Doudna JA, Nogales E (2005) Structural roles for human translation factor eIF3 in initiation of protein synthesis. *Science* **310:** 1513–1515

Trachsel H, Erni B, Schreier MH, Braun L, Staehelin T (1979) Purification of seven protein synthesis initiation factors from Krebs II ascites cells. *Biochim Biophys Acta* **561:** 484–490

Unbehaun A, Borukhov SI, Hellen CU, Pestova TV (2004) Release of initiation factors from 48S complexes during ribosomal subunit joining and the link between establishment of codon–anticodon base-pairing and hydrolysis of eIF2-bound GTP. *Genes Dev* **18:** 3078–3093

Valasek L, Mathew AA, Shin BS, Nielsen KH, Szamecz B, Hinnebusch AG (2003) The yeast eIF3 subunits TIF32/a, NIP1/c, and eIF5 make critical connections with the 40S ribosome in vivo. *Genes Dev* **17:** 786–799

Valasek L, Nielsen KH, Hinnebusch AG (2002) Direct eIF2-eIF3 contact in the multifactor complex is important for translation initiation in vivo. *EMBO J* **21:** 5886–5898

Valasek L, Nielsen KH, Zhang F, Fekete CA, Hinnebusch AG (2004) Interactions of eukaryotic translation initiation factor 3 (eIF3) subunit NIP1/c with eIF1 and eIF5 promote preinitiation complex assembly and regulate start codon selection. *Mol Cell Biol* **24:** 9437–9455

Valasek L, Phan L, Schoenfeld LW, Valaskova V, Hinnebusch AG (2001) Related eIF3 subunits TIF32 and HCR1 interact with an RNA recognition motif in PRT1 required for eIF3 integrity and ribosome binding. *EMBO J* **20:** 891–904

Valasek L, Trachsel H, Hasek J, Ruis H (1998) Rpg1, the *Saccharomyces cerevisiae* homologue of the largest subunit of mammalian translation initiation factor 3, is required for translational activity. *J Biol Chem* **273:** 21253–21260

Verlhac MH, Chen RH, Hanachi P, Hershey JW, Derynck R (1997) Identification of partners of TIF34, a component of the yeast eIF3 complex, required for cell proliferation and translation initiation. *EMBO J* **16:** 6812–6822

Vornlocher HP, Hanachi P, Ribeiro S, Hershey JW (1999) A 110-kilodalton subunit of translation initiation factor eIF3 and an associated 135-kilodalton protein are encoded by the *Saccharomyces cerevisiae* TIF32 and TIF31 genes. *J Biol Chem* **274:** 16802–16812

Yahalom A, Kim TH, Winter E, Karniol B, von Arnim AG, Chamovitz DA (2001) *Arabidopsis* eIF3e (INT-6) associates with both eIF3c and the COP9 signalosome subunit CSN7. *J Biol Chem* **276:** 334–340

Yen HC, Chang EC (2000) Yin6, a fission yeast Int6 homolog, complexes with Moe1 and plays a role in chromosome segregation. *Proc Natl Acad Sci USA* **97:** 14370–14375

Zhou C, Arslan F, Wee S, Krishnan S, Ivanov AR, Oliva A, Leatherwood J, Wolf DA (2005) PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. *BMC Biol* **3:** 14