

Addendum

Arabidopsis eIF3e interacts with subunits of the ribosome, Cop9 signalosome and proteasome

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The roles of individual Eukaryotic translation Initiation Factor 3 (eIF3) subunits are largely unclear, though some are essential, while others are thought to have regulatory roles. The “e” subunit, also known as Int-6, is a candidate for a regulatory subunit as it is not essential for translation initiation in yeasts. To further elucidate the roles of eIF3e, we have employed an interaction-trap screen using the yeast two-hybrid system. eIF3e interacts in yeast with subunits of the ribosome, COP9 signalosome and 26S proteasome. These interactions mesh well with our recent results which showed that eIF3e is degraded in a CSN-dependent, proteasome-dependent fashion, and inhibits translation when present in excess.

eIF3 is by far the largest of the generic translation initiation complexes.¹ eIF3 stimulates binding of the ternary complex (eIF2, Met-tRNA^{Met}, GTP) to the 40S ribosome, inhibits premature association of 40S and 60S subunits, and may serve as a bridge between eIF4G and the 40S ribosome during the early stage of initiation.^{2,3} Recent studies support the idea that one or more translational regulatory pathways impinge on eIF3.^{2,4} From a plant point of view, this also is likely true. The eIF3i subunit has a role in the BRI1 receptor kinase signaling pathway.⁵ During seedling development, wheat eIF3 subunits accumulate in an asynchronized fashion,⁶ consistent with temporally dispensable and perhaps regulatory roles of some of its subunits, and the activator of ribosome shunting of cauliflower mosaic virus, TAV, interacts with eIF3g.⁷ Arabidopsis eIF3h is dispensable for basal translation, but essential for translation of specific transcripts carrying multiple upstream open reading frames in their 5' leader.⁸

The e subunit, eIF3e, has several characteristics that make it an excellent candidate for a regulatory subunit.⁹ eIF3e is a cytoplasmic protein, yet is also detected in the nucleus in many organisms

including plants.¹⁰⁻¹³ The role of nuclear eIF3e is still unclear, though it has been associated with (i) control of 26S proteasome activity,¹⁴ (ii) the COP9 signalosome (CSN),¹¹ another regulator of proteolysis,^{15,16} (iii) the ‘nuclear speckle’ proto-oncogene products Rfp and PML,^{10,17} and (iv) spindle organization.^{18,19} Although eIF3e is not essential for global translation initiation in yeasts (*S. pombe*),²⁰⁻²² there is good evidence that eIF3e plays a pivotal role in translation.^{20,23,24} In fission yeast, two different eIF3 complexes were detected, one containing and one lacking eIF3e. These two eIF3 complexes may be associated with different mRNAs.²¹

eIF3e Interacts with Ribosome, Proteasome and Cop9 Signalosome Subunits

To identify proteins that interact with eIF3e, we have undertaken an interaction-trap screen using the yeast two-hybrid assay. Since eIF3e fused to LexA activates the *LacZ* reporter gene by itself (not shown), two partial eIF3e constructs, encoding the N-terminal 292 amino acids (*eIF3e-N*), and the C-terminal 153 amino acids that contains the PCI domain (*eIF3e-C*), each fused to LexA, were used as baits. These fusion proteins do not activate the reporter genes. *eIF3e-N* or *eIF3e-C*, each cloned as a *LexA* fusion, was transformed in yeast cells together with a cDNA expression library extracted from a six-day-old light-grown Arabidopsis seedlings.²⁵ Following screening of 1.6×10^6 and 6×10^9 yeast colonies for *eIF3e-N* and *eIF3e-C* respectively, seven and 152 independent putative interacting clones were identified as leucine auxotrophs and showed β -galactosidase activity strictly dependent on galactose. The putative eIF3e interacting proteins were retransformed back into the original yeast strain to ensure that they do not activate the reporter genes by themselves, or with a non-specific bait, and characterized by restriction analyses.

The eIF3e interactors are detailed in Table 1. Of particular relevance for our work, the amino terminal half of eIF3e interacts with the S9 protein subunit of the 40S ribosome small subunit, while the PCI-carboxyl terminus interacted with RPN12a, the Arabidopsis homologue of subunit 12 of the 19S regulatory particle of the 26S proteasome.²⁶ Both clones interact with CSN1, CSN4 and CSN6. The eIF3-PCI construct also interacts with CSN8, while the N-terminus construct also interacts with CSN7, consistent with our earlier work.¹¹ Both the eIF3e amino terminus and the PCI containing clones interact with proteins whose genes have yet to be studied.

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Table 1 eIF3e interacting proteins

Clone	At #/common name	Function
eIF3e-N interactors	40S ribosomal protein S9	Subunit S9 of the 40S ribosomal small subunit
	At1g27930	Unknown
	CSN1	Cop9 Signalosome subunit 1
	CSN4	Cop9 Signalosome subunit 4
	CSN6	Cop9 Signalosome subunit 6
	CSN7	Cop9 Signalosome subunit 7
eIF3e-C interactors	RPN12	Non ATPase subunit of the 19S regulatory complex from 26S proteasome
	At4g30620	Unknown
	CSN1	Cop9 Signalosome subunit 1
	CSN4	Cop9 Signalosome subunit 4
	CSN6	Cop9 Signalosome subunit 6
	CSN8	Cop9 Signalosome subunit 8

Relevance of Identified Interactions

These interactions results fit in well with our recently published analysis of eIF3e in Arabidopsis.²⁷ First, the interaction between the N-terminal part of eIF3e and S9 of the 40S ribosomal small subunit are consistent with the negative role that we showed for eIF3e in translational regulation. In vitro translation assays were inhibited when exogenous eIF3e was added to the reaction mixture, but not when another eIF3 subunit, eIF3b, was added. Induced overexpression of *eIF3e* in transgenic Arabidopsis seedlings led to a decrease in ³⁵S-Met incorporation, and a reduction in polysome peaks, coupled with an increase in non-polysomal RNAs, further illustrating that excess eIF3e can inhibit translation. Based on the eIF3e-S9 interaction, we speculate that the translational inhibition partly arises from excess eIF3e competing with binding of the full eIF3 complex to the 40S ribosome.²⁸

Second, the interactions with numerous CSN subunits clearly solidify the eIF3e-CSN physical connection. We previously reported that full length eIF3e copurifies with CSN from cauliflower and directly binds the Arabidopsis CSN7 in yeast, in vitro and *in planta*.^{11,29} Mammalian eIF3e also interacts with CSN7, as well as CSN6, but not with CSN5,³⁰ lending further support to the interactions between Arabidopsis eIF3e and CSN subunits described here.

In Yahalom et al.,²⁷ we provided the biological context for the eIF3e-CSN interaction. Degradation of eIF3e by the proteasome is dependent on the CSN. Mutants in CSN subunits accumulate high levels of eIF3e protein, while *eIF3e* transcript levels are actually reduced. This then meshes with the phenotypic overlap between the *cop* mutants in the CSN and the *eIF3e*-overexpressing transgenic lines. This overlap includes *cop* phenotypes in dark-grown seedlings, developmental arrest of light-grown seedlings, problems in flower development similar to *ufo* mutants, and reduction in translation rates. These results clearly indicate that eIF3e and CSN function in the same developmental pathways, and identifies translational control as a new target for CSN-based activity.

Third, and perhaps less clear, is the interaction between eIF3e-C and RPN12. While this interaction clearly meshes earlier studies showing a physical connection between eIF3e and the proteasome.^{14,30} However, the biological significance of this interaction is still up in the air. While Yen et al.,¹⁴ reported that the role of eIF3e

in binding RPN5 in *S. pombe* is to regulate its nuclear import, which affects the degradation of specific cell cycle regulators, it is also conceivable, that the interaction of eIF3e with the proteasome arises from eIF3e being a proteasome substrate.²⁷

References

- Burks EA, Bezerra PP, Le H, Gallie DR, Browning KS. Plant initiation factor 3 subunit composition resembles mammalian initiation factor 3 and has a novel subunit. *J Biol Chem* 2001; 276:2122-31.
- Siridechadilok B, Fraser CS, Hall RJ, Doudna JA, Nogales E. Structural roles for human translation factor eIF3 in initiation of protein synthesis. *Science* 2005; 310:1513-5.
- Hinnebusch AG. eIF3: a versatile scaffold for translation initiation complexes. *Trends Biochem Sci* 2006; 31:553-62.
- Holz MK, Ballif BA, Gygi SP, Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* 2005; 123:569-80.
- Ehsan H, Ray WK, Phinney B, Wang X, Huber SC, Clouse SD. Interaction of Arabidopsis BRASSINOSTEROID-INSENSITIVE 1 receptor kinase with a homolog of mammalian TGF-beta receptor interacting protein. *Plant J* 2005; 43:251-61.
- Gallie DR, Le H, Tanguay RL, Browning KS. Translation initiation factors are differentially regulated in cereals during development and following heat shock. *The Plant Journal* 1998; 14:715-22.
- Park HS, Himmelbach A, Browning KS, Hohn T, Ryabova LA. A plant viral "reinitiation" factor interacts with the host translational machinery. *Cell* 2001; 106:723-33.
- Kim TH, Kim BH, Yahalom A, Chamovitz DA, von Arnim AG. Translational regulation via 5' mRNA leader sequences revealed by mutational analysis of the Arabidopsis translation initiation factor subunit eIF3h. *Plant Cell* 2004; 16:3341-56.
- von Arnim AG, Chamovitz DA. Protein Homeostasis: A Degrading Role for Int6/eIF3e. *Curr Biol* 2003; 13:323-5.
- Desbois C, Rousset R, Bantignies F, Jalinot P. Exclusion of Int-6 from PML nuclear bodies by binding to the HTLV-I Tax oncoprotein. *Science* 1996; 273:951-3.
- Yahalom A, Kim TH, Winter E, Karniol B, von Arnim AG, Chamovitz DA. Arabidopsis eIF3e (INT-6) associates with both eIF3c and the COP9 signalosome subunit CSN7. *J Biol Chem* 2001; 276:334-40.
- Watkins SJ, Norbury CJ. Cell cycle-related variation in subcellular localization of eIF3e/INT6 in human fibroblasts. *Cell Proliferation* 2004; 37:149-60.
- Guo J, Sen GC. Characterization of the interaction between the interferon-induced protein P56 and the Int6 protein encoded by a locus of insertion of the mouse mammary tumor virus. *J Virol* 2000; 74:1892-9.
- Yen HC, Gordon C, Chang EC. Schizosaccharomyces pombe Int6 and Ras homologs regulate cell division and mitotic fidelity via the proteasome. *Cell* 2003; 112:207-17.
- Chamovitz DA, Glickman MH. Quick guide: The COP9 signalosome. *Curr Biol* 2002; 12:232.
- Chamovitz DA, Wei N, Osterlund MT, von Arnim AG, Staub JM, Matsui M, Deng XW. The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* 1996; 86:115-21.
- Morris-Desbois C, Bochar V, Reynaud C, Jalinot P. Interaction between the Ret finger protein and the Int-6 gene product and co-localisation into nuclear bodies. *J Cell Sci* 1999; 112:3331-42.
- Yen HC, Chang EC. Yin6, a fission yeast Int6 homolog, complexes with Moe1 and plays a role in chromosome segregation. *Proc Natl Acad Sci USA* 2000; 97:14370-5.
- Morris C, Jalinot P. Silencing of human Int-6 impairs mitosis progression and inhibits cyclin B-Cdk1 activation. *Oncogene* 2005; 24:1203-11.

20. Bandyopadhyay A, Matsumoto T, Maitra U. 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* 2000; 11:4005-18.
21. Zhou C, Arslan F, Wee S, Krishnan S, Ivanov AR, Oliva A, Leatherwood J, Wolf DA. PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. *BMC Biol* 2005; 3:14.
22. Shalev A, Valasek L, Pise-Masison CA, Radonovich M, Phan L, Clayton J, He H, Brady JN, Hinnebusch AG, Asano K. *Saccharomyces cerevisiae* protein Pci8p and human protein eIF3e/Int-6 interact with the eIF3 core complex by binding to cognate eIF3b subunits. *J Biol Chem* 2001; 276:34948-57.
23. Bandyopadhyay A, Lakshmanan V, Matsumoto T, Chang EC, Maitra U. 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* 2002; 277:2360-7.
24. Asano K, Merrick WC, Hershey JW. 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* 1997; 272:23477-80.
25. Kwok SF, Staub JM, Deng XW. Characterization of two subunits of Arabidopsis 19S proteasome regulatory complex and its possible interaction with the COP9 complex. *J Mol Biol* 1999; 285:85-95.
26. Smalle J, Kurepa J, Yang P, Babychuk E, Kushnir S, Durski A, Vierstra RD. Cytokinin growth responses in Arabidopsis involve the 26S proteasome subunit RPN12. *Plant Cell* 2002; 14:17-32.
27. Yahalom A, Kim TH, Roy B, Singer R, Von Arnim AG, Chamovitz DA. Arabidopsis eIF3e is regulated by the COP9 signalosome and impacts development and protein translation. *Plant J* 2008; in press.
28. Chaudhuri J, Chakrabarti A, Maitra U. Biochemical characterization of mammalian translation initiation factor 3 (eIF3). Molecular cloning reveals that p110 subunit is the mammalian homologue of *Saccharomyces cerevisiae* protein Prt1. *J Biol Chem* 1997; 272:30975-83.
29. Karniol B, Yahalom A, Kwok S, Tsuge T, Matsui M, Deng XW, Chamovitz DA. The Arabidopsis homologue of an eIF3 complex subunit associates with the COP9 complex. *FEBS Lett* 1998; 439:173-9.
30. Hoareau Alves K, Bochar V, Rety S, Jalinot P. Association of the mammalian proto-oncogene Int-6 with the three protein complexes eIF3, COP9 signalosome and 26S proteasome. *FEBS Lett* 2002; 527:15-21.