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Article 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,[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 x 10^6 and 6 x 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 eIF3e-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 interaction results fit 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 is 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 ^{35}S-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.

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. 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]

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