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Protein Homeostasis: A Degrading Role for Int6/eIF3e

Dispatch

Albrecht G. von Arnim[1] and Daniel A. Chamovitz[2]

Similarities between the three related 'PCI' complexes — eIF3, the COP9 signalosome and the proteasome lid — have hinted at novel pathways controlling protein homeostasis. Recent experiments with fission yeast have begun to weigh in with genetic evidence.

The name of a gene product can be misleading, masking its true function. Such may be the case for a 'schizophrenic' protein that is variously known as the 'e' subunit of eukaryotic translation initiation factor 3, eIF3e, or 'Int6' after a preferred chromosomal integration site for the mouse mammary tumor virus (MMTV). Recent work by Yen *et al.* [1] in fission yeast suggests a novel and surprising role for eIF3e/Int6 in regulating protein turnover through binding to the regulatory lid of the 26S proteasome. These results are intriguing because fission yeast eIF3e/Int6 appears to function within a Ras1-mediated pathway governing cytoskeleton assembly and mitotic chromosome segregation. If these results hold true for mammalian cells, they might explain the perplexing tumorigenic properties of this janus-headed translation initiation factor.

eIF3e/Int6 has been identified independently at least five times (reviewed in [2]). It is a conserved 48 kDa protein that has been recognized in most eukaryotes, with the notable exception of budding yeast, *Saccharomyces cerevisiae*, which has a related protein called Pci8p [3]. eIF3e/Int6 has a carboxy-terminal 'PCI' domain, versions of which have been found in subunits of three complexes: the proteasome regulatory lid, the COP9 signalosome (CSN) and eIF3 (Figure 1). Integration of MMTV into the mouse *Int6* gene generates gain-of-function alleles, which are tumorigenic [4]. These results are rather puzzling, as Int6 persistently copurifies with eIF3 [5–7]. In contrast to other eIF3 subunits, however, eIF3e/Int6 is not essential for translation in fission yeast [7,8], although the eIF3 complex is unstable in an *eIF3e/int6* mutant [9]. These and other data suggest that eIF3e/Int6 is a regulatory subunit of eIF3. In keeping with this notion, human eIF3e/Int6 may be the target for an antiviral translational repression pathway [10].

As outlined below, however, evidence has also been accumulating that eIF3e/Int6 has a darker side. Chang and coworkers [1] first noticed that fission yeast *eIF3e/int6* mutants have phenotypes similar to proteasome lid mutants. For example, they are hypersensitive to the arginine analog canavanine, which increases the demand for proteasome activity by causing protein misfolding. They accumulate ubiquitinated proteins, suggesting proteasome malfunction. Furthermore, the *eIF3e/int6* mutation, rather benign on its own, enhances numerous other mutations in proteasome subunits. This genetic interaction is indicative of a physical interaction, as eIF3e/Int6 co-purifies with various proteasome components, including the regulatory lid, the base, and the catalytic core. So had Int6 first been isolated this way, it would have a third name related to the proteaseome!

Interestingly no enhancement was observed between mutations of eIF3e/Int6 and those of the lid subunit Rpn5, suggesting that Rpn5 is the lid subunit that mediates the effect of eIF3e/Int6. Remarkably, eIF3e/Int6 and Rpn5 physically interact in yeast two-hybrid and pulldown assays. Overproduction of Rpn5 partially rescues an *eIF3e/int6* mutation, but not *vice versa*, suggesting that eIF3e/Int6 targets Rpn5 rather than the other way around. Several defects in mutant fission yeast are rescued by human eIF3e/Int6, indicating conservation of function [1].

So what is a translation factor doing binding the proteasome? In all fairness, it should be pointed out that an eIF3–proteasome connection was noted earlier in fission yeast and mammalian cells, although its significance was perhaps not fully appreciated [11–13]. What makes the work of Yen *et al.* [1] unique is that it questions whether and how the interaction might affect proteasome activity. One obvious place to look would be subcellular localization, for eIF3e/Int6 has nuclear import and export activity, while the fission yeast proteasome is concentrated near the inner nuclear envelope. True to hypothesis, in the *eIF3e/int6* mutant background, Rpn5 is generally localized to the cytoplasm, while Int6 localization is not affected by an *rpn5* mutation. This suggests that one role for eIF3e/Int6 may be to 'piggy-back' Rpn5 into the nucleus, where it assembles into the proteasome.

Among the polyubiquitinated proteins that accumulate in the *int6* mutant are a mitotic cyclin (Cdc13) and securin (Cut2) [1]. Cdc13 accumulation blocks cytokinesis and exit from mitosis, while Cut2 impairs sister-chromatid separation. Accumulation of these two proteins may thus be partly responsible for the abnormally lengthy mitosis and inefficient chromosome segregation observed in *eIF3e/int6* mutant cells. Moreover, eIF3e/Int6 appears to be involved in the cell polarity and cytoskeleton responses orchestrated by the Rho-like G protein Cdc42 downstream of the G protein Ras1 [14] (Figure 2). Int6 interacts indirectly with a guanine-nucleotide exchange factor for Cdc42, termed Scd1 [1]. If these data apply to mammalian cells, they might start to explain why in breast cancer, as opposed to most cancers, up-regulation of Ras correlates positively with a favorable prognosis [1].

The bridge between Scd1 and eIF3e/Int6 is provided by yet another eIF3 subunit, eIF3d/Moe1 [15]. eIF3d/Moe1 also co-purifies with Rpn5 in the presence of Yin6 [1]. Moreover, *eIF3d/moe1* and *eIF3e/int6* mutants have related microtubule defects in

[1]Department of Botany, The University of Tennessee, Knoxville, Tennessee 37996, USA. [2]Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel.
E-mail: dannyc@tauex.tau.ac.il

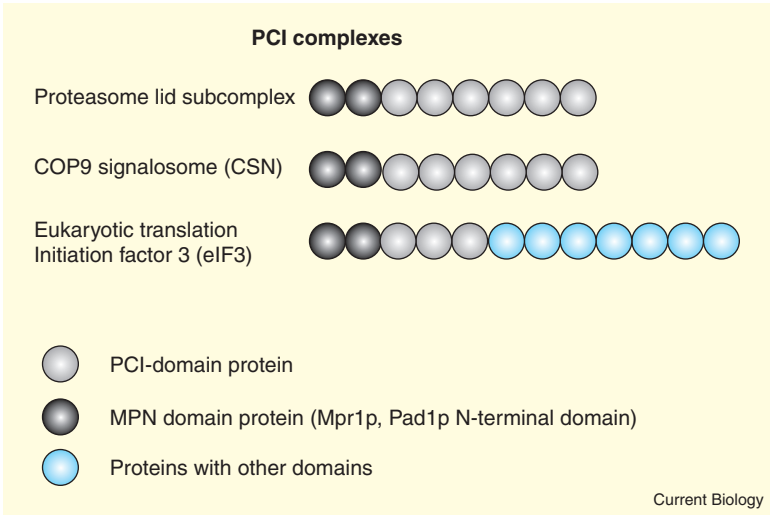


Figure 1. The three PCI complexes are structurally and evolutionarily related.

The proteasome lid subcomplex regulates access of ubiquitinated proteins to the 26S proteasome. The COP9 signalosome (CSN) regulates E3 ubiquitin ligases and various kinases. eIF3 facilitates the loading and subsequenct scanning of the 40S ribosomal subunit along the 5′ leader of mRNAs.

the Ras1-mediated chromosome segregation pathway [14]. This is where the picture becomes complicated. Whereas Yen *et al.* [1] found proteasome association of eIF3e/Int6, Dunand-Sauthier *et al.* [13] found such association specifically for eIF3i/Sum1 and not for eIF3e/Int6. Dunand-Sauthier *et al.* [13] further reported that, under stress conditions, eIF3e/Int6 and other eIF3 subunits relocalize to cytoplasmic granules rather than to the proteasome [13].

These observations could be reconciled if eIF3e/Int6 localization was specifically regulated by external conditions. The nucleocytoplasmic shuttling activity of eIF3e/Int6, as well as its effect on localization of eIF3d/Moe1 and Rpn5, might be taken as circumstantial evidence for this idea. How eIF3e/Int6's subcellular localization affects translation and protein turnover remains to be clarified by dedicated experimentation. For example, it is not known whether eIF3e interacts with the proteasome exclusively in the nucleus.

What is so tantalizing about the crosstalk between eIF3 and the proteasome lid is that the two complexes are evolutionarily related both to each other and to the CSN. A CSN–proteaseome connection has been long proposed [16] and the eIF3–CSN connection has also been gaining acceptance [12,17]. There is still more to eIF3e/Int6 than meets the eye. In budding yeast, the eIF3 complex lacks an eIF3e-like subunit, but there is a recognizable eIF3e/Int6 ortholog, Pci8p, which interacts with a CSN-like protein complex. Mutations in this complex or in Pci8p cause the same biochemical defect observed in CSN-deficient mutants of other species [18,19], giving further credence to the CSN–eIF3e

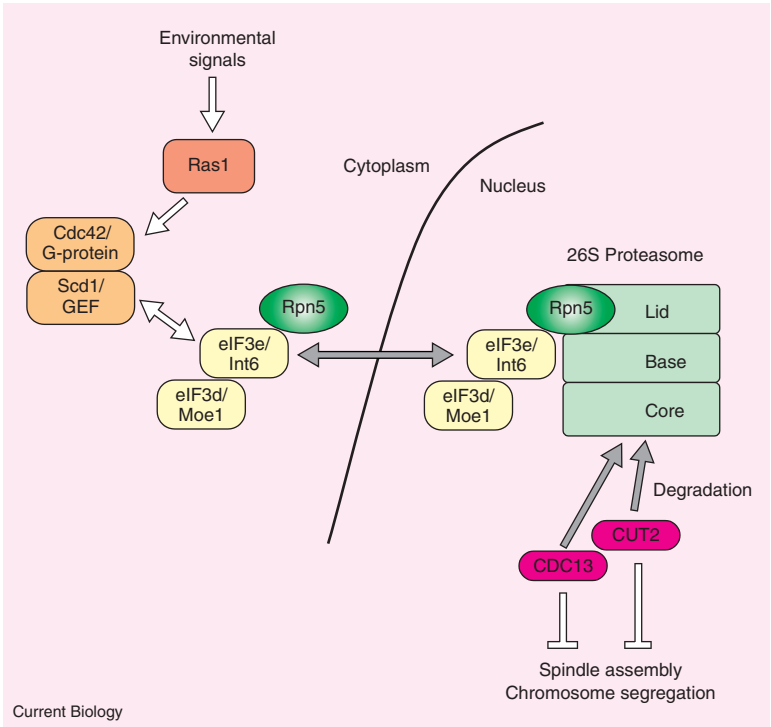
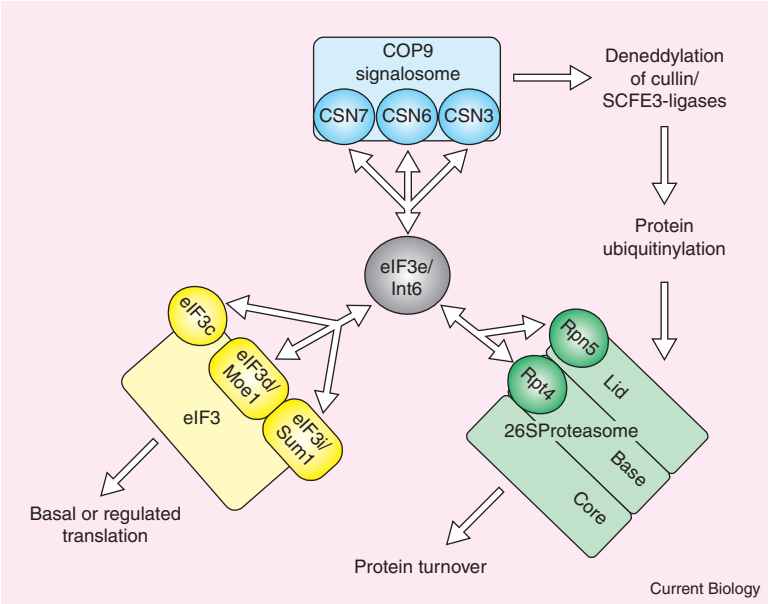


Figure 2. Possible function of eIF3e/Int6 in fission yeast.

eIF3e/Int6 may function in fission yeast as a component of a Ras1 triggered signaling pathway, in part by regulating proteolytic activity through an interaction with the proteasome lid subunit Rpn5.

Figure 3. Interactions of eIF3e/Int6.

eIF3e/Int6 interacts with components from the three PCI complexes, perhaps to regulate their activities, or to mediate between them.



connection shown previously in *Arabidopsis* and in mammalian cells [12,17]. But the new data [1] provide the first genetic evidence for a functional interaction between subunits of the three PCI complexes.

From an evolutionary perspective, perhaps eIF3e/Int6 began as an accessory to the CSN, and was later co-opted by the other PCI complexes. One testable model deriving from this work is that eIF3e/Int6 regulates all three complexes by modulating specific subunits (Figure 3). Thus, eIF3e/Int6 might be necessary for the deneddylation of specific CSN substrates, for the translation of specific transcripts by eIF3, and for the degradation of specific targets by the proteasome. Alternatively, eIF3e/Int6 may help coordinate the action of the three PCI complexes. For example, it is plausible that stress conditions that cause protein misfolding during translation may also attract components of the protein turnover machinery into the vicinity of the translation apparatus. Local elevation of the concentration of these PCI complexes might be mediated by proteins, such as eIF3e/Int6, that shuttle between the different complexes.

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