Elongator function in tRNA wobble uridine modification is conserved between yeast and plants

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Summary

Based on studies in yeast and mammalian cells the Elongator complex has been implicated in functions as diverse as histone acetylation, polarized protein trafficking and tRNA modification. Here we show that Arabidopsis mutants lacking the Elongator subunit AtELP3/ELO3 have a defect in tRNA wobble uridine modification. Moreover, we demonstrate that yeast elp3 and elp1 mutants expressing the respective Arabidopsis Elongator homologues AtELP3/ELO3 and AtELP1/ELO2 assemble integer Elongator complexes indicating a high degree of structural conservation. Surprisingly, in vivo complementation studies based on Elongator-dependent tRNA nonsense suppression and zymocin TNAse toxin assays indicated that while AtELP1 rescued defects of a yeast elp1 mutant, the most conserved Elongator gene AtELP3, failed to complement an elp3 mutant. This lack of complementation is due to incompatibility with yeast ELP1 as coexpression of both plant genes in an elp1 elp3 yeast mutant restored Elongator’s tRNA modification function in vivo. Similarly, AtELP1, not ScELP1 also supported partial complementation by yeast–plant Elp3 hybrids suggesting that AtElp1 has less stringent sequence requirements for Elp3 than ScElp1. We conclude that yeast and plant Elongator share tRNA modification roles and propose that this function might be conserved in Elongator from all eu karyotic kingdoms of life.

Introduction

Studies in fields as disparate as leaf development (Nelis sen et al., 2003; 2005) and drought resistance (Chen et al., 2006) in plants, neurodegeneration in humans (Anderson et al., 2001; Slaugenhaupt et al., 2001), cytotoxicity of a fungal toxin (Frohloff et al., 2001) and transcription elongation (Otero et al., 1999) have surprisingly converged on a conserved protein complex termed Elongator. The protein complex is composed of two subcomplexes with subunits Elp1, Elp2, and Elp3 as well as Elp4, Elp5 and Elp6 forming the hexameric holo-Elongator (Petrakis et al., 2004). Elp3 has a GNAT-type histone acetyltransferase (HAT) domain and has also been named Kat9 K-acetyltransferase to conform with recent acetyltransferase nomenclature (Allis et al., 2007). The Elongator complex was initially identified in yeast to interact with hyperphosphorylated RNA polymerase II and implicated in chromatin modification during transcription elongation (Otero et al., 1999; Wittschieben et al., 1999; Kristjuhan et al., 2002; Winkler et al., 2002; Gilbert et al., 2004; Kristjuhan and Svejstrup, 2004).

Surprisingly, it turned out that yeast Elongator mutants defective in any of the Elongator subunit genes (ELP1 to ELP6) are lacking tRNA modifications at wobble uridines or thiouridines in position 34 of the anticodon (Huang et al., 2005). One of these tRNA modifications, 5-methoxycarbonyl-methyl-2-thiouridine (mcm 5s2U), renders Saccharomyces cerevisiae sensitive to a toxin (zymocin) secreted by Kluuyveromyces lactis (reviewed in Schaffrath and Breunig, 2000) and leads to the identification of ELP/TOT genes in a screen for zymocin-resistant mutants (Frohloff et al., 2001). The γ-subunit (γ-toxin) of the zymocin is a tRNA endonuclease that cleaves a subset of tRNAs carrying the modified wobble uridine (mcm 5s2U) (Huang et al., 2005; Lu et al., 2005; 2008; Jablonowski et al., 2006).
Genomic sequence analysis suggests that an Elongator complex exists in all eukaryotes. In plants, Elongator mutants have been isolated in a screen for mutants with aberrant leaf morphology. The Arabidopsis thaliana elong-gata (elo) class of leaf mutants consisting of four loci, elo1 to elo4, is characterized by narrow and elongated shape of leaves and deficiencies in organ growth. ELO1 identified the Arabidopsis homologue of yeast ELP4, ELO2 is homologous to ELP1, and ELO3 to ELP3 (Nelissen et al., 2005). Moreover, the DEFORMED ROOTS AND LEAVES (DRL1) gene is allelic to elo4 and shows sequence similarity to yeast KTI12, which encodes an Elongator partner protein (Fichtner et al., 2002a; Nelissen et al., 2003). The similar phenotypes of these mutants support the view that the gene products function in a complex and suggest that plant Elongator is similar to the one in yeast.

So far the molecular function of the complex is not well defined. Apparently, its acetyltransferase activity is essential but the substrate spectrum may be wider than initially anticipated (Svejstrup, 2007). Phenotypes associated with Elongator deficiency range from impaired zygotic paternal genome demethylation (Okada et al., 2010) to altered microtubule dynamics in human and Caenorhabditis elegans (Creppe et al., 2009; Solinger et al., 2010). Recently, mammalian Elongator was linked to acetylation of α-tubulin and shown to affect the migration and differentiation of cortical neurons (Creppe et al., 2009). In C. elegans Elongator deficiency was also associated with neurological and developmental defects and tRNA modification defects (Chen et al., 2009). C. elegans mutants still contain acetylated α-tubulin although the level may be reduced (Chen et al., 2009; Solinger et al., 2010). It has also been questioned whether histones are primary substrates for the acetyltransfer reaction because transcription-related phenotypes in yeast Elongator mutants could be suppressed by tRNA overexpression (Esberg et al., 2006).

Whether protein acetylation is the primary and only biochemical activity of Elongator in eukaryotes is unknown. The pleiotropic phenotypes of yeast Elongator mutants in processes as diverse as translation, exocytosis, filamentous growth and transcriptional silencing (Rahl et al., 2005; Johansson et al., 2008; Abdullah and Cullen, 2009; Li et al., 2009) might be explained by consequential effects of improper tRNA modification or by multiple acetylation substrates for Elongator, but there is also evidence for additional biochemical activities (Huang et al., 2005; Li et al., 2009; Lipardi and Paterson, 2009).

Here we have addressed the question whether the tRNA modification function is conserved between yeast and plants. We show that in an A. thaliana elo3 mutant, tRNA wobble uridine modifications including mcms2U are compromised. By complementing yeast elo mutants with A. thaliana ELO/AtELP genes we demonstrate that the yeast subunits can assemble with plant polypeptides to form hybrid Elongator complexes indicating high structural similarity between yeast and plant Elongator. Strikingly, despite the fact that AtElp3/ELO3, the most conserved subunit, could structurally replace yeast Elp3 functional complementation with AtELP3/ELO3 was not observed unless ELP1 was simultaneously replaced by AtELP1/ELO2. Taken together the data strongly support the view that the tRNA modification function of Elongator is conserved between yeast and plants and most likely among all eukaryotes.

Results

Formation of a yeast Elongator complex containing AtELP3

The A. thaliana genome contains one and only one homologue for each of the yeast ELP genes and evidence for a similar hexameric complex, composed of two subcomplexes, was recently obtained by tandem affinity purification (Nelissen et al., 2010). We tried to complement yeast elo mutant strains with the corresponding plant cDNAs fused to a yeast promoter. Sensitivity to γ-toxin, the active component of the K. lactis killer toxin zymocin provided a sensitive assay for Elongator function. If the heterologous protein integrated into the yeast Elongator complex and functioned in restoring tRNA modification, we expected reversion of the toxin resistance phenotype of the Elongator mutant. Because the AtELP3 subunit is most similar to its yeast homologue, we first tried to complement the elo3 mutant. A c-myc-tagged version of the AtELP3 protein could be produced at levels comparable to those of yeast ELP3-c-myc (see below), but the toxin resistance of the yeast elo3A mutant was unaffected by the AtELP3-c-myc gene (not shown) or an untagged AtELP3 allele (Fig. 1A). Likewise, thermostability and hypersensitivity to caffeine, additional phenotypes of Elongator mutants, were not altered by the plant gene (Fig. 1B). Reintroduction of the yeast ELP3 gene into the elo3A reporter, however, fully complemented all three phenotypes (Fig. 1A and B).

The failure of plant AtELP3 to substitute for yeast ELP3 function was not due to instability of the protein as AtELP3-c-myc was precipitated from total yeast protein extracts at levels comparable to ELP3-c-myc (Fig. 1C, top panel). c-myc-tagged ELP3 and AtELP3 both could co-precipitate the largest Elongator subunit ELP1 indicating interaction between AtELP3 and ELP1 (Fig. 1C, bottom panel).

To analyse whether AtELP3-Elp1 interaction occurred in the context of the Elongator complex we made use of the fact that interaction between the subunits Elp5 and ELP2 depends on the structural integrity of the complex and the presence of ELP3 (Frohloff et al., 2003; Petrakis et al., 2004). We constructed elo3Δ reporter strains expressing

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a c-myc-tagged version of Elp2 and an HA-tagged version of Elp5. As expected, co-immunoprecipitation of Elp2-c-myc with Elp5-HA was not observed when Elp3 was lacking (Fig. 2A, lane 4) but was found when the elp3Δ mutant was complemented with the yeast ELP3 gene on a plasmid (Fig. 2A, lane 3). When AtELP3 or AtELP3-c-myc alleles were introduced instead of ELP3, pull-down of Elp2-c-myc by Elp5-HA was less efficient (Fig. 2A, lanes 5 and 6), but significantly higher than in the empty vector control (Fig. 2A, lane 4). Thus, plant AtELP3 promotes Elp5-Elp2 interaction in a yeast elp3Δ mutant background.

Using the same approach we also tested whether the chimeric complex was able to interact with Kti12, a protein that associates with the Elongator complex (Fichtner et al., 2002a; Petrakis et al., 2005). As with Elp5-HA, Kti12-HA was able to pull-down Elp2-c-myc in the presence but not in the absence of yeast Elp3 (Fig. 2B, lanes 3 and 4) and again AtELP3 could substitute for yeast Elp3 in this interaction assay (Fig. 2B, lanes 5 and 6). The latter finding is particularly intriguing because Elp2–Kti12 interaction relies on the association between the two Elongator subcomplexes Elp1–Elp2–Elp3 and Elp4–Elp5–Elp6 (Fichtner et al., 2002b; Frohloff et al., 2003). We conclude...
that AtELP3 can structurally replace Elp3 in the yeast Elongator complex but apparently the complex is not functional.

**Plant AtELP1 restores Elongator subunit interactions in an elp1Δ yeast mutant**

Incorporation of AtElp3 into yeast Elongator requires interaction with much less conserved components. Elp1 and AtELP1 display only 19% amino acid identity compared with 67% between Elp3 and AtELP3. To analyse whether AtELP1 could also structurally replace yeast Elp1 in the complex, an elp1Δ strain that contained the epitope-tagged Elongator subunits Elp3-HA and Elp2-c-myc was transformed with an AtELP1 cDNA clone. Consistent with previous reports (Frohloff et al., 2003; Petrakis et al., 2004), we found that the Elp1 subunit is not only required for Elp3-Elp2 interaction (Fig. 3A, lane 2) but also for stability of yeast Elp3. Hence, an HA-tagged version of Elp3 was not detectable in total protein extracts of the elp1Δ mutant (Fig. 3A, bottom panel, lane 2). Reintroduction of the yeast ELP1 gene restored Elp3-HA stability and interaction between Elp2 and Elp3 (Fig. 3A, lane 4). Remarkably, the same held true when AtELP1 was introduced (Fig. 3A, lane 3). Expression of the plant gene from the inducible GAL1 promoter in the elp1Δ background allowed Elp2-c-myc to precipitate Elp3-HA. AtELP1 expression also restored the interaction between Elp2 and Kti12 (Fig. 3B).

Again, as in the AtELP3-expressing elp3 mutant, the efficiency of subunit interactions was somewhat reduced compared with that of the ELP1 transformants. Nonetheless, our data show that AtELP1 like AtELP3 are assembled into complexes where they are able to structurally replace the respective yeast Elongator subunits.

**Together, plant AtELP1 and AtELP3 support tRNA modification in yeast**

Because Elp3 requires Elp1 for stability, its function may depend on specific contacts between these two proteins explaining the failure of AtELP3 to functionally complement the elp3Δ mutant in combination with yeast Elp1. To address this possibility we tested whether AtELP3 might function in yeast when supplied with AtELP1. FLAG-AtELP1 was expressed together with the AtELP3-c-myc gene in a yeast elp1Δ elp3Δ double mutant. FLAG-AtELP1 could be detected in the anti-c-myc precipitates together with AtELP3-c-myc (Fig. 4A). Like in the single mutants, interaction between the epitope-tagged subunits Elp5-HA and Elp2-c-myc (Fig. 4B) as well as Kti12-HA and Elp2-c-myc (Fig. 4C) could be restored in the double mutant by the simultaneous expression of untagged (lanes 5) or tagged (lanes 6) versions of AtELP1 and AtELP3. Elp2-c-myc (lanes 3, 5 and 6) and AtELP3-c-myc (lane 6) could be detected in the anti-HA precipitates of strains containing yeast (lane 3) or *A. thaliana* Elp1 and Elp3. We conclude that a chimeric yeast–plant Elongator complex can form under these conditions. Intriguingly, AtELP1 and AtELP3 together were able to restore γ-toxin sensitivity in the yeast elp1Δ elp3Δ double mutant indicating that the chimeric Elongator complex was functional (Fig. 5A). Because toxin sensitivity requires the Elongator-dependent tRNA wobble uridine modification mcm5s2U (Lu et al., 2005), the *A. thaliana* subunits can reasonably be assumed to carry out a function in tRNA...
To support this conclusion, we used a second assay monitoring Elongator-dependent tRNA modification that is based on the SUP4 suppressor tRNA gene (Huang et al., 2005). The SUP4 allele codes for a UAA ochre suppressor tRNA\text{\textsuperscript{\textgamma}} with a G34-to-U34 exchange in the anticodon. Suppression of ochre nonsense codons by SUP4 requires Elongator-dependent wobble uridine modification (Huang et al., 2005). In an elp1\textDelta elp3\textDelta double deletion strain (CMY160) carrying the ochre mutation ade2-1 the SUP4 tRNA suppressor was inactive resulting in an Ade\textsuperscript{-} phenotype (Fig. 5B, vector control). Transformation with two plasmids encoding either yeast Elp1 and Elp3 or AtElp1 and AtElp3 restored SUP4 tRNA suppression, allowing for ade2-1 readthrough and growth on adenine-deficient medium. Although growth in the latter case was weak we conclude that the AtElp1-AtElp3 containing chimeric Elongator can functionally replace yeast Elongator and promote ochre suppression by SUP4. Yeast Elp1 with AtElp3 was inactive in the adenine growth assay (data not shown) as in the g-toxin assay (Fig. 5A).

Elp3 contains two protein motifs that are found in other functional contexts, the C-terminal GNAT-like HAT domain related to Gcn5 histone acetyltransferase (Wittschieben et al., 1999) and a central domain found in radical S-adenosyl-L-methionine enzymes (radical SAM domain) (Chinenov, 2002; Paraskevopoulou et al., 2006). To test which part of AtElp3 is not compatible with ScElp1 we constructed two hybrid variants with the HAT domain of yeast Elp3 replaced by that of AtELP3 and vice versa (Fig. 5C). Like AtELP3, both chimeric Elp3 variants were unable to complement the elp1 elp3 double mutant in the presence of yeast Elp1 (data not shown). However, in combination with AtELP1, AtELP3 and both hybrid proteins allowed for weak growth on adenine-deficient medium.

**Fig. 4.** Formation of a chimeric Elongator complexes composed of yeast and plant subunits.

A. AtELP1 and AtELP3 interact with each other in yeast. Anti-c-myc immunoprecipitates of elp1\textDelta elp3\textDelta strains (CMY134) containing FLAG-AtELP1 (pJET13) and AtELP3-c-myc were probed with anti-c-myc antibodies to detect AtELP3-c-myc and with anti-FLAG-antibodies to detect FLAG-AtELP1. Immunoprecipitates with anti-c-myc antibodies from elp1\textDelta elp3\textDelta cells containing pFLAG-AtELP1 (lane 2) or no epitope-tag (lane1) served as negative controls.

B. AtELP1 and AtELP3 restore Elp2–Elp5 interaction in the elp1\textDelta elp3\textDelta mutant. Protein extracts from cells expressing HA-tagged Elp5 together with c-myc-tagged Elp2 in an elp1\textDelta elp3\textDelta double mutant background (CMY305) and transformed with plasmids containing ELP1 (pFF13), ELP3 (pFF9), AtELP1 (pDJ98), AtELP3 (YEpA4), FLAG-AtELP1 (pJET13), AtELP3-c-myc (pELO3-myc) and vector controls (YEplac181, YEplac195) were immunoprecipitated using anti-HA antibodies. The antibodies used to detect the indicated proteins by Western blotting are marked on the right. Protein extracts from cells without epitope-tag and cells expressing only AtELP3-c-myc served as negative controls. Pre-IPs served as loading control (bottom panel).

C. AtELP1 and AtELP3 restore Elp2–Kti12 interaction. Protein extracts from strain CMY302 expressing HA-tagged Kti12 together with c-myc-tagged Elp2 in an elp1\textDelta elp3\textDelta background and transformed (see B) were analysed as described in (B).
medium indicative of SUP4-dependent ochre suppression of the ade2-1 mutation (Fig. 5B). Apparently, AtELP1 has a less stringent requirement with respect to Elp3 sequence than ScElp1. The weak Ade+ phenotype in combination with AtELP1 but not with yeast ELP3 was highly reproducible; however, we could not confirm reconstitution of tRNA modification by the hybrid Elp3 variants using the γ-toxin assay (data not shown). Probably partial complementation results in hypomodification of tRNAs and gives zymocin resistance.

tRNA modification is affected in Elongator-deficient plant cells

Restoration of ochre suppression and γ-toxin sensitivity of the yeast elp1Δ elp3Δ double mutant by AtELP1 and AtELP3 suggested that plant Elongator may also affect tRNA modification in plant cells. For all but one tRNA modification genes of yeast (TRM genes) homologues can be found in plant genomes suggesting that plant tRNAs are modified in a similar way. To test whether Elongator deficiency in A. thaliana had any impact on tRNA modification RNA preparations enriched for small stable RNA were compared between wild-type and homozygous elo3 mutants. RNA was isolated from leaves of several independent plants, degraded to nucleosides and analysed by HPLC. The elution profiles were very similar for both samples except for the parts shown in Fig. 6, where 5-carbamoyl-methyluridine (ncm5U) (Fig. 6A and B) and mcm5s2U (Fig. 6C and D) eluted. Both modified uridine nucleosides (compare Fig. 6E) were lacking in the sample from the elo3 mutant. These data clearly show that the elo3 mutation prevented the ncm5 and mcm5s2-uridine modifications from being generated supporting the view that plant Elongator is involved in tRNA modification in a way similar to its yeast homologue. This indicated that the role of Elongator in tRNA modification might be conserved among lower and higher eukaryotes.

Discussion

Biochemical characterizations of yeast and human Elongator and genomic sequences have indicated that the subunit composition of this complex is conserved among eukaryotes (reviewed in (Svejstrup, 2007)). The data presented in this paper reinforce this view and provide evi-


dence for a remarkable structural conservation between plant and yeast Elongator complexes. We further demonstrate that an A. thaliana Elongator mutant is lacking the very same tRNA modifications previously shown in yeast to be Elongator-dependent (Huang et al., 2005). Together with similar deficiencies reported for C. elegans Elongator mutants (Chen et al., 2009), these data strongly support the view that tRNA modification might be a function of Elongator that is conserved in animals, fungi and plants.

A crucial question is how Elongator affects tRNA modification. The acetyltransferase activity of the Elp3 subunit has been shown to be required (Huang et al., 2005). One possibility is that biosynthesis or activity of the enzymes generating the ncm^5 or mcm^5 side chains on the tRNA wobble uridines depend on acetylation. Alternatively, Elongator may directly participate in the tRNA modification reaction. The Elongator subunits Elp1 and Elp3 could be specifically cross-linked to tRNA^Glu^ but not tRNA^Met^ (Huang et al., 2005) supporting the latter model.

A radical mechanism based on the putative radical SAM domain flanking the HAT motif in Elp3 (Chinenov, 2002; Paraskevopoulou et al., 2006) might be involved.

Fig. 6. AtELP3/ELO3 is required for formation of mcm^5s^2U and ncm^5U modified nucleosides in tRNA.
A.–D. Total tRNA isolated from wild-type and elo3 plants was analysed by HPLC (see Experimental procedures). Wild-type profiles are shown on the left, elo3 profiles in the right panels.
A. and B. The parts of the chromatograms between retention times 15.5 and 19.6 min are displayed. The arrow in (B) indicates the expected retention time of ncm^5U. Chromatograms were monitored at 254 nm.
C. and D. The parts of the chromatograms between retention times 40.0 and 52.5 min are displayed. The arrow in (D) indicates the expected retention time of mcm^5s^2U. Chromatograms were monitored at 314 nm.
E. Uridine modifications found at the wobble position of eukaryotic tRNAs. Elongator-dependent side groups are circled (Huang et al., 2005).
We have used yeast as a tool to analyse whether AtELP3 was able to function in the context of a heterologous Elongator complex. Two sensitive assays that allow to monitor the Elongator-dependent tRNA modification were used. The first one detects mcm5's2U-tRNA<sup>At</sup> and, to a lesser extent, mcm5's2U-tRNA<sup>Ve</sup> and mcm5's2U-tRNA<sup>Sm</sup>. These tRNAs are the primary targets of the tRNA endonuclease activity associated with the K. lactis zymocin. Defects in formation of the modified uridine and typical of Elongator mutants cause zymocin resistance (Lu et al., 2005; 2008; Jablonowski et al., 2006; Studte et al., 2008). Restoration of zymocin sensitivity in resistant mutants indicates efficient formation of the modified tRNAs. The second assay makes use of the SUP4 ochre suppressor tRNA, which also depends on the mcm5 modification function of Elongator (Huang et al., 2005). Both assays are sensitive to a point mutation in the HAT domain of yeast Elp3 (Frohloff et al., 2001; Huang et al., 2005). Under conditions of partial complementation the two assays are expected to differ in sensitivity. Hypomodified tRNAs would confer (some degree of) resistance in the zymocin assay (i.e. the mutant phenotype) by escaping endonucleolytic cleavage but might support some growth on selective plates (the wild-type phenotype) in the nonsense suppression assay.

We have shown here that a double mutant lacking Elp3 and Elp1 can be complemented by the Arabidopsis homologues AtELP1 and AtELP3/EL03, which together restore zymocin sensitivity and SUP4-mediated nonsense suppression. Thus, these two subunits cooperate to functionally replace yeast Elp1 and Elp3 indicating that they have a similar role in the plant Elongator complex.

Surprisingly, despite 67% sequence identity between ScElp3 and AtELP3, the latter was unable to complement the yeast elp3 mutation. Only in combination with AtELP1 could complementation of the Elongator mutant phenotypes by the AtELP3 genes be achieved. AtELP3-c-myc alone was incorporated into the yeast complex as shown by its ability to restore association between the large and small subcomplexes and between Elongator and its partner protein Kti12. Both types of interactions require Elp3 and the structural integrity of the complex (Frohloff et al., 2001; Fichtner et al., 2002a; Fichtner et al., 2003; Petrikis et al., 2005). We conclude that, although an Elongator complex in which AtELP3 replaces Elp3 appears structurally intact, the AtELP3 containing chimeric complex is unable to provide the activity required for tRNA modification.

The lack of functional complementation by AtELP3 is probably not due to the reduced efficiency of Elp2–Elp5 or Elp2–Kti12 interactions as the same reduction was observed in the AtELP3 and AtELP1 containing chimeric complex, which is functional. AtELP3 appears stable in yeast in the presence of ScElp1 excluding the possibility that lack of function is due to protein degradation, which is observed for ScElp3 in the absence of ScElp1 (Fig. 3).

To determine whether sequence divergence in the HAT domain was responsible for the lack of complementation, domain swapped variants with ScHAT replaced by AtHAT and vice versa were tested. Like AtELP3, both variants were inactive in the presence of ScELP1. This failure of the AtHAT to replace the corresponding yeast domain contrasts with the ability of the HsELP3-HAT to do so (Li et al., 2005). However surprisingly, in the SUP4 tRNA suppression assay (but not in the zymocin assay), both hybrid ELP3 genes were able to partially complement the yeast elp3 mutation in combination with AtELP1. This indicates weak U34-tRNA modification mediated by AtELP1 in combination with multiple structural variants of Elp3 including ScElp3 (Chen et al., 2006). AtELP1 appears more tolerant towards sequence variations in the Elp3 protein. The crucial role of ScElp1 phosphorylation/ dephosphorylation (Mehlgarten et al., 2009) might be important in this context. Alternatively but less likely, AtELP1 may contribute to complementation in yeast via an additional function not found in ScElp1, like the RNA-dependent RNA polymerase activity recently reported for Drosophila Elp1 (Lipardi and Paterson, 2009).

A current challenge is to understand the biological function(s) of the Elongator complexes in the various organisms and their possible divergence during evolution. If, as proposed here and supported by studies in C. elegans (Chen et al., 2009), tRNA modification defects are a general consequence of Elongator deficiency in eukaryotes it will be essential to determine whether the mutant phenotypes are causally related to this defect. Studies in yeast support such a view. Pleiotropic phenotypes of yeast Elongator mutants including hypoaecetylation of histones, delayed adaptation of transcription to changing environmental conditions as well as delocalization of the secretory protein Sec2 could all be suppressed by over-expression of two tRNAs, tRNA<sup>Ve</sup> and tRNA<sup>Sm</sup>, both of which in wild-type cells undergo Elongator-dependent anticodon modifications (Esberg et al., 2006). Based on these data it has been proposed that the effects of Elongator mutants on transcription and secretion are indirect consequences of influences on translation caused by inappropriate tRNA modification. Apparently, U34 modifications in the anticodon increase the decoding efficiency of A- and G-ending codons (Yokoyama et al., 1985; Lim, 1994; Durant et al., 2005; Esberg et al., 2006; Begley et al., 2007; Johansson et al., 2008). Thus, the translation of individual proteins might be differentially affected depending on their amino acid composition and codon usage. Moreover, yeast elp mutants are synthetic lethal with mutations affecting thiolation of U34 (Esberg et al., 2006; Johansson et al., 2008) emphasizing the importance of the wobble uridine modifications.
### Yeast strains

| Strain          | Description                                                                 | Source/reference                          |
|-----------------|-----------------------------------------------------------------------------|-------------------------------------------|
| *K. lactis*     | **AWJ137** α leu2 trp1 [k1 k2] killer and zymocin producer                  | Karin D. Breunig                           |
| *S. cerevisiae* | **FY1679-08A** MATα ura3-52 leu2-1 trp1::S16 his3::HIS20 GAL                | Euroscaf                                   |
|                 | **CMY307** as FY1679-08A, but elp3::natNT2                                    | This study                                 |
|                 | **FFY3** as FY1679-08A, but ELP3-(cmyc)3::SpHIS3                              | Frohloff et al. (2001)                    |
|                 | **CMY306** as FY1679-08A, but elp1::TRP1, elp3::natNT2                        | This study                                 |
|                 | **FFY2/3-dt** as FY1679-08A, but ELP2-(cmyc)3::SpHIS3, ELP3-(HA)3::KITRP1    | Fichtner et al. (2002a)                   |
|                 | **FFY2/3-dt-1d** as FY23-dt, but elp1::XIURA3                                 | Fichtner et al. (2002b)                   |
|                 | **FFY2/4dt** as FY1679-08A, but ELP2-(cmyc)3::SpHIS3, KT122-(HA)3::KITRP1    | Fichtner et al. (2002a)                   |
|                 | **CMY300** as FFY2/4dt, but elp1::hphNT1                                     | This study                                 |
|                 | **CMY301** as FFY2/4dt, but elp3::natNT2                                     | This study                                 |
|                 | **CMY302** as FFY2/4dt, but elp1::hphNT1, elp3::natNT2                       | This study                                 |
|                 | **FFY2/5dt** as FY1679-08A, but ELP2-(cmyc)3::SpHIS3, ELP5-(HA)3::KITRP1     | Frohloff et al. (2003)                    |
|                 | **CMY304** as FFY2/5dt, but elp3::natNT2                                     | This study                                 |
|                 | **CMY305** as FFY2/5dt, but elp1::hphNT1, elp3::natNT2                       | This study                                 |
|                 | **W303-1a** MATα ade2-1 his3-11, 15 leu2-3, -112 trp1-1 ura3-1 can1-100      | Laboratory stock                          |
|                 | **CMY135** as W303, but elp3::natNT2                                        | This study                                 |
|                 | **CMY134** as W303, but elp1::hphNT1, elp3::natNT2                          | This study                                 |
|                 | **UMY2893** MATα SUP4 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15   | Huang et al. (2005)                       |
|                 | **UMY2916** MATα SUP4 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15, 3 | Huang et al. (2005)                       |
|                 | **CMY160** as UMY2916, but elp1::HIS3                                        | This study                                 |

A causal relationship between tRNA hypomodification and Elongator phenotypes does not exclude that Elongator has additional activities or multiple targets for the acetyltransferase reaction. In plants, yeast and human cells the complex is also found in the nucleus and its acetyltransferase reaction. In plants, yeast and human tor has additional activities or multiple targets for the Elongator phenotypes does not exclude that Elongator in transcription elongation of specific genes, and at least two of these genes, SHY2/IAA3 and LAX2, were also affected in histone H3K14 acetylation, which is a predominant target of the ScELP3-HAT (Nelissen et al., 2010). The data were taken as evidence for a role of Elongator in transcription elongation of specific genes, which may explain the observed influences on cell proliferation and development in elo mutants. How specificity for these genes might be achieved is currently unknown. Because auxin signalling is specific to plants whereas neuron development is specific to animals studies on the role of Elongator in these diverse processes might reveal how a protein complex with (a) conserved molecular function(s) has evolved to fulfil these kingdom specific functions. We believe that the answer to these questions can be found at the cellular level.

Taken together, our data indicate that the Elongator complexes are structurally and functionally highly conserved between yeast and plants, such that yeast subunits can be replaced by their plant orthologues. This offers the opportunity to take advantage of the yeast model system in structure–function studies of Elongator complexes from other kingdoms. It is likely that eukaryotic Elongator complexes have a conserved biochemical activity. Based on reconstitution of U34 tRNA modification in yeast by plant Elongator subunits and the requirement of ATElp3/ELO3 for this modification in plants, we favour the view that this conserved activity is directly related to tRNA modification.

#### Experimental procedures

**Yeast strains, media and general methods**

Yeast strains used are listed in Table 1. Yeast was grown in rich media containing yeast extract, peptone and 2% dextrose (YPD) or 2% galactose (YPG) or on synthetic complete medium (SC) (Sherman, 1991). Thermosensitivity was assayed on YPD medium at 30 or 38°C for 2–3 days. Drug sensitivity was assayed at 30°C with 5 mM caffeine. ade2-1 ochre stop codon suppression by the SUP4 gene was tested as described (Huang et al., 2005). Yeast was transformed with plasmid DNA or polymerase chain reaction (PCR) products according to a previous protocol (Schiestl and Gietz, 1989). Primers are listed in Table S1.

#### Arabidopsis thaliana strains and growth conditions

An *A. thaliana* homozygous T-DNA insertion mutant in At5g50230 (*ATELP1*) derived from GABI-Kat Line 636H02
and the corresponding wild type (Columbia ecotype) were analysed for tRNA modification. Total RNA was prepared from leaves of several independent plants grown under short day conditions.

**Plasmid constructions**

pFF9, a YEplac195-based plasmid carrying ELP3 has been described (Frohloff et al., 2001). *Arabidopsis* AtELP3/ELP3 was amplified from cDNA clone RAFL0811J12 (http://www.brc.riken.jp) with primers cDNAfw and cDNA2re inserting flanking Sal restriction sites. The Sal fragment was fused to the yeast ADH1 promoter and integrated into YEplac195 to give YEpA4. The AtELP3 open reading frame was verified by DNA sequencing. In yatELP3 the ADH1 promoter and 5’ end of the AtELP3 gene from YEpA4 was replaced after cleavage with HindIII and Agel by the yeast ELP3 promoter and 5’ end using primers E3′-fw and E3′-re and pFF9 as template. yatELP3M is a triple c-myc-tagged variant of yatELP3. pFF13 is a YEplac181-based plasmid, carrying the yeast ELP3 gene (Fichtner et al., 2002a). pDJ98 is a multicopy plasmid for galactose-regulated expression of the AtELP1 gene carrying the selection markers URA3 and leu2d (Chen et al., 2006). pJET13, carrying FLAG-AtELP1 under control of the GAL1 promoter was obtained by PCR amplification of the coding sequence from pFLAG-ELO2 (Z. Gong, unpublished) and cloned into Sal-cleaved pCM22. In the latter, the GAL1 promoter was introduced on a EcoRI-BamHI fragment from plasmid pRB1438 (a kind gift from Mike Stark, University of Dundee, UK) into YEplac181 (LEU2). pCM22 served also as destination vector to clone untagged AtELP1 from pDJ98 via Sal restriction sites to obtain pCM26 for the SUP4 suppression assay. The centromeric plasmid p424TDH (Mumberg et al., 1995) served as the destination vector for ELP3 variants in double complementation studies. p424TDH-ScElp3myc encodes a C-terminal triple c-myc-tagged variant of ScELP3 (kindly provided by O. Onuma, Univ. Halle), p424TDH-AtElp3myc a C-terminal triple c-myc-tagged variant of AtELP3. PCR-mediated domain swap of the AtELP3 HAT domain (amino acids 436–565) into p424TDH-ScElp3myc (1–426) and of ScElp3-HAT (382–557) into p424TDH-AtElp3myc (1–390) resulted in plasmids pELP3-ATHAT and pAtELP3-ScHAT respectively.

**Yeast genetic manipulations**

Defined elp1Δ and elp3Δ null alleles and genetic variants encoding hemagglutinin (HA) or c-myc epitope-tagging proteins were obtained after transformation of PCR fragments generated with template plasmids containing the marker genes YDP-KIU (URA3), YDP-SpH (HIS3), pFA6a-aphNT1, pFA6a-natNT2 (for deletions) or pYM3 and pYM5 (for HA and c-myc epitope-tagging) (Knop et al., 1999; Frohloff et al., 2001; Jablonowski et al., 2001; Janke et al., 2004). Manipulations were verified by PCR and by killer eclipse assays (Kishida et al., 1996) to test for biological functionality.

**Elongator complementation studies in yeast**

To analyse the function of Arabidopsis ELO2 and ELO3 encoded gene products, the γ-toxin sensitivity was assayed (Frohloff et al., 2001). In detail, strains CMY135 (elp3Δ) and CMY134 (elp1Δ elp3Δ) were transformed with pDJ98 (ELO2/AtELP1), YEpA4 (ELO3/AtELP3), pFF13 (ELP1) and pFF9 (ELP3) (Frohloff et al., 2001; Fichtner et al., 2002a; Chen et al., 2006) or the respective empty vector controls. Subsequently, plasmid pHMS14 (Frohloff et al., 2001) expressing the γ-toxin subunit of *K. lactis* zymocin under the GAL1 promoter was introduced into the transformed strains. Strains were grown on 2% (w/v) glucose SC medium under selective conditions and 10-fold serial dilutions were spotted on glucose and galactose medium. The response to γ-toxin induction was monitored on galactose plates after 3 to 4 days at 30°C.

**Immunological techniques**

Detection of tagged proteins used anti-c-myc (A-14) and anti-HA (F-7) (Santa Cruz) antibodies. Anti-Elp1 antibodies (Otero et al., 1999; Wittschieben et al., 1999) were kindly provided by Dr J. Svejstrup (London Research Institute, Cancer Research, UK). An anti-FLAG antibody (Sigma–Aldrich) was used for detection of FLAG-ELO2. Protein concentrations were determined by the method of Bradford (Bradford, 1976). Antibody cross-linking to protein A-Sepharose, preparation of protein extract and co-immunoprecipitations were performed as described previously (Zachariae et al., 1996; Frohloff et al., 2001).

**tRNA isolation and HPLC analysis**

tRNA was prepared from total RNA preparations as described (Bjork et al., 2001). Purified tRNA was digested with Nuclease P1 for 16 h at 37°C and then treated with bacterial alkaline phosphatase for 2 h at 37°C. The hydrolysate was analysed by high-pressure liquid chromatography with a Develosil C-30 reverse-phase column as described (Gehrke et al., 1982; Gehrke and Kuo, 1990).

**Acknowledgements**

The authors would like to thank Osita Onuma for providing plasmid p424TDH-ScElp3myc, Jesper Q. Svejstrup for anti-Elp1 antibodies, Jens-Eike-Täubert and Sabrina Zink for help
and advice and Mieke van Lijsebettens for discussion and communication of results prior to publication. We thank Sabine Rosahl (Institute of Plant Biochemistry, Halle) for providing the Arabidopsis thaliana T-DNA insertion line. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (DFG) in SFB 648 ‘Molekulare Mechanismen der Informationsverarbeitung in Pflanzen’ to K.D.B and R.S. and by the Sachsen-Anhalt federal graduate programme ‘Structures and Mechanisms of Biological Information Processing’. In addition, R.S. acknowledges support by the BBSRC (BB/F019106/1). A.B. is supported by grants from the Swedish Cancer Foundation (07 0637), Swedish Research Council (621-2006-4269) and Bernhard and Signe Bäckström Foundation (223-438-07).

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