Recent Advances in the Role of the Elongator Complex in Plant Physiology and tRNA Modification: A Review

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Abstract

The Elongator complex is a multifunction protein complex which has been shown to be involved in transcriptional elongation, DNA replication and repair, tubulin and histone acetylation, gene silencing and transfer RNA uridine modification. The composition of the Elongator complex is found to be highly conserved in eukaryotes, protein homologs of various subunits have been identified in fungi, plant, animal, and human. Remarkably, mutation in genes encoding the Elongator complex structural components all results in defects of transfer RNA wobble uridine modification, and this function of the Elongator complex is also conserved in eukaryotes. The Elongator complex mutants in higher plants have pleiotropic phenotypes including defects in vegetative growth, abscisic acid hypersensitivity, elevated tolerance to drought and oxidative stress. What is the relationship between the Elongator complex’s function in nucleoside modification and its activity in other cellular pathways? This review summarizes the recent advances in study of function of the Elongator complex, in the aspects of cell physiology and molecular biology.

Key words: the Elongator complex, transfer RNA, nucleoside modification

INTRODUCTION

From the first report of the Elongator complex in 1999 (Otero et al. 1999) from Saccharomyces cerevisiae, protein homologs of various subunits have been found in various eukaryotic systems including Caenorhabditis elegans, Arabidopsis thaliana, Drosophila melanogaster, and Homo Sapiens (Hawkes et al. 2002). The most striking feature of all is the structural conservation of the protein complexes, as well as the phenotype similarity resulting from loss-of-function mutation in any of the protein subunits. With analogy to the elongation-factors in translation, the Elongator complex associates with RNA polymerase II (RNA pol. II) during transcription process. The strong physical interaction between the Elongator complex and RNA pol. II has been shown by in vitro immunoprecipitation assay, and this interaction relies on the hyper-phosphorylated status of the CTD domain of RNA pol. II (Jablonowski et al. 2001b).

However, after the initial discovery, the Elongator complex have been suggested to participate in diverse cellular pathways, including histone modification/ acetylation (Wittschieben et al. 1999), exocytosis (Rahl et al. 2005), tubulin acetylation (Creppe et al. 2009), response to DNA damage (Li et al. 2009), transcriptional silencing (Li et al. 2009), and tRNA nucleoside modification (Huang et al. 2005; Esberg et al. 2006).
The dysfunction of the Elongator complex proteins in *D. melanogaster* and *C. elegans* result in defect of embryo development (Chen et al. 2009; Walker et al. 2011), and several neural degenerative diseases have been associated with different alleles coding for the Elongator complex subunits (Anderson et al. 2001; Crolla and van Heyningen 2001; Kleinjan et al. 2002; Strug et al. 2009). Most of these phenotypes have been attributed to translational defect, for instance neural cells are particularly sensitive to translational defects due to their high demand of protein synthesis, and also over-expression of certain tRNA isoacceptors harbouring the affected nucleoside can partially rescue the phenotype (Chen et al. 2011).

So far all mutants in the structural components of the Elongator complex lead to specific tRNA nucleoside modification defects at position 34 (wobble position), which harbours xm^3^U (including ncm^3^U: 5-carboxomethylmethyluridine and mcm^3^U: methoxy carbonylmethyl-uridine) type of uridine modifications in *S. cerevisiae* (Huang et al. 2005), *C. elegans* (Chen et al. 2009) and *A. thaliana* (Mehlgarten et al. 2010). The connection between the Elongator complex’s role in tRNA wobble uridine modification and metabolic and physiological duties is still a mystery. In this review, we focus on the recent advance in the Elongator complex function in higher plants, particular associated with tRNA uridine nucleoside modifications.

**STRUCTURAL COMPOSITION OF THE ELONGATOR COMPLEX AND FUNCTIONAL ASSOCIATION**

The Elongator complex is composed of six protein subunits, the corresponding protein and their molecular mass are: Elp1-150, Elp2-90, Elp3-60, Elp4-50, Elp5-35, and Elp6-30 kDa (Otero et al. 1999; Winkler et al. 2001, Table). Elp1-3 form the core complex, the Elp4-6 subcomplex forms a hetero-hexameric ring-like structure which is essential for the binding of anticodon stem-loop of substrate tRNAs (Fig., Glatt et al. 2012). Contrary to its primary role in transcriptional elongation and histone modification, the subcellular localization of the Elongator complex subunits are mainly cytoplasmic, except for the catalytic subunit Elp3 (Fichtner et al. 2002b; Creppe et al. 2009; Miśkiewicz et al. 2011).

The largest subunit of the Elongator complex is the Elp1 protein, which can be phosphorylated. Actually the phosphorylation status of Elp1 is regulated by several proteins including Sit4 and Sit4-associated proteins-Sap185 and Sap190 (Jablonsowski et al. 2001a, 2004), Kti11-Kti14 (Mehlgarten et al. 2009). Elp3 is the functional centre of the Elongator complex, the histone acetyl transferase (HAT) activity for histone modification directly links the chromatin structure deformation with elevated transcription activity mediated by RNA pol. II. The presence of an iron-sulfur cluster on Elp3 protein allows for the binding of S-AdoMet (Paraskevopoulos et al. 2006). The radical shoot apical meristem (SAM) activity of Elp3 was indicated to be involved in DNA methylation/demethylation at specific cytidine positions in paternal zygotic cells (Okada et al. 2010). The sub-complex Elp4-6 all share a same RecA like protein fold but without the ATPase consensus sequence, and it has been shown in vitro that Elp4-6 could hydrolyse ATP and use this reaction to bind tRNA. The hexameric NTPase structure is common to other nucleic acids-binding proteins such as Rho GTPase (Glatt et al. 2012).

The proper function of the Elongator complex need the collaboration with other proteins, among all Kti12 is the most tightly related. Both genetic and biochemical evidence suggest there is considerable functional overlap between Kti12 and the Elongator complex (Frohloff et al. 2001; Petrakis et al. 2005), Kti12 could physically interact with Elp3 and Elp5 proteins, however deletion of *KTI12* does not influence the assembly of the Elongator complex. Kti12 is an ancient ATP/GTP binding protein which has also been found in *Achaea Methanopurus kandleri* (Fichtner et al. 2002a). All Kti12 homologs contain conserved P-loop motif, but the plant Kti12 protein also contains a calmodulin binding domain at the C-terminus (Nelissen et al. 2005). Kti14 protein belongs to casein kinase family, it has been suggested for post-translational regulation of the Elongator complex’s function (Mehlgarten and Schaffrath 2003). Kti14 could bind the Elongator complex in the presence of Kti12 (Fichtner et al. 2003; Mehlgarten et al. 2009), however no physical interaction has been shown between Kti11 or Kti13 protein.
with the Elongator complex (Fichtner and Schaffrath 2002c). Genetic evidence points to a negative regulation of Kti11 on Kti12 (Fichtner and Schaffrath 2002c); whereas Kti13 acts as a positive regulator by serving as a potential guanosine-exchange-factor (GEF) for Kti12. Kti13 belongs to RCC1 protein family involved in chromatin-remodelling and condensation (Fichtner and Schaffrath 2002c). Finally, protein interactions between Elp1-Elp2, Elp2-Kti12, Elp2-Kti13 and Kti12-Kti13 need the presence of Elp3 (Fichtner et al. 2002b), emphasizing the important role of Elp3 both for structural integrity and function of the Elongator complex.

PHENOTYPE OF elp AND kti MUTANTS IN YEAST AND HIGHER PLANTS SUGGESTS FUNCTIONAL CONSERVATION

Zymocin resistance in yeast

The original identification of KTI genes was from isolation of killer-toxin-insensitive S. cerevisiae towards

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Table Structural and regulatory components of the Elongator complex

<table>
<thead>
<tr>
<th>Gene</th>
<th>Annotation</th>
<th>Mutant phenotype</th>
<th>Mutant modification</th>
<th>References</th>
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<tbody>
<tr>
<td>ELP1</td>
<td>Elongator core subunit</td>
<td>Yeast: slow growth, G1 cell cycle delay, Ts, zymocin resistance, calcofluor, and 6-AU sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Fellows (2001); Frohloff et al. (2001); Anderson et al. (2001); Huang et al. (2005); Nelissen et al. (2005); Chen et al. (2006); Zhou et al. (2009); Nelissen et al. (2010); Chen et al. (2009)</td>
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<tr>
<td>ELP2</td>
<td>Elongator core subunit</td>
<td>Yeast: slow growth, G1 cell cycle delay, Ts, zymocin resistance, calcofluor, and 6-AU sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Fellows (2001); Frohloff et al. (2001); Huang et al. (2005); Zhou et al. (2009)</td>
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<tr>
<td>ELP3</td>
<td>Elongator core subunit</td>
<td>Yeast: slow growth, G1 cell cycle delay, Ts, zymocin resistance, calcofluor, and 6-AU sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Fellows (2001); Frohloff et al. (2001); Huang et al. (2005); Nelissen et al. (2005); Melhergarten et al. (2010); Nelissen et al. (2005); Nelissen et al. (2010); Xu et al. (2012); Chen et al. (2009); Walker et al. (2011)</td>
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<tr>
<td>ELP4</td>
<td>Elongator sub-complex</td>
<td>Arabidopsis: narrow leaf and reduced root growth, accumulation of anthocyanin, ABA hypersensitivity, tolerant to oxidative stress, reduced apical dominance</td>
<td>Lack ncm/U, mcm/U</td>
<td>Huang et al. (2005); Nelissen et al. (2005); Zhou et al. (2009); Nelissen et al. (2010)</td>
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<td>ELP5</td>
<td>Elongator sub-complex</td>
<td>Yeast: zymocin resistance, slow growth, G1 cell cycle delay, Ts, calcofluor, and 6-AU sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Frohloff et al. (2001); Huang et al. (2005)</td>
</tr>
<tr>
<td>ELP6</td>
<td>Elongator sub-complex</td>
<td>Arabidopsis: narrow leaf and reduced root growth, accumulation of anthocyanin, ABA hypersensitivity, tolerant to oxidative stress</td>
<td>Lack ncm/U, mcm/U</td>
<td>Huang et al. (2005); Zhou et al. (2009)</td>
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<td>KTI11</td>
<td></td>
<td>Yeast: G1 cell cycle arrest, zymocin resistance</td>
<td>Lack ncm/U, mcm/U</td>
<td>Fichtner et al. (2002c); Huang et al. (2005)</td>
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<tr>
<td>KTI12</td>
<td>ATP/GTP binding</td>
<td>Yeast: zymocin resistance, slow growth, G1 cell cycle delay, Ts, calcofluor and 6-AU sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Frohloff et al. (2001); Fichtner et al. (2002a); Huang et al. (2005); Nelissen et al. (2003); Nelissen et al. (2005)</td>
</tr>
<tr>
<td>KTI13</td>
<td></td>
<td>Yeast: G1 cell cycle arrest, zymocin resistance</td>
<td>Lack ncm/U, mcm/U</td>
<td>Fichtner et al. (2002c); Huang et al. (2005)</td>
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<tr>
<td>KTI14</td>
<td></td>
<td>Yeast: G1 cell cycle arrest, zymocin resistance, calcofluor, and 6-AU sensitive, MMS sensitive</td>
<td>Lack ncm/U, mcm/U</td>
<td>Mehlergarten et al. (2003); Huang et al. (2005)</td>
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<td>TRM9</td>
<td></td>
<td>Yeast: zymocin resistant, paranycin sensitive</td>
<td>Lack mcm/U</td>
<td>Jablowski et al. 2006; Kallhor and Clarke (2003); Huang et al. (2008); Leitme et al. (2011)</td>
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<tr>
<td>SIT4</td>
<td></td>
<td>Yeast: G1 cell cycle arrest, zymocin resistance, 6-AU sensitive, Ts</td>
<td>Lack ncm/U, mcm/U</td>
<td>Huang et al. (2008); Jablowski et al. (2001a)</td>
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<tr>
<td>SAP185,190</td>
<td></td>
<td>Yeast: G1 cell cycle arrest, zymocin resistance, 6-AU sensitive, Ts</td>
<td>Lack ncm/U, mcm/U</td>
<td>Jablowski et al. (2001a)</td>
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zymocin (Fichtner and Schaffrath 2002c; Frohloff 2001), which is secreted by *Kluyveromyces lactis* to inhibit the growth of other sensitive yeast (Butler et al. 1991, 1994). Most strikingly *kti11-14* mutants in *S. cerevisiae* all shared zymocin-resistant phenotypes as the *elp* mutants (Mehlgarten and Schaffrath 2003), the data supported the idea of zymocin as a general translation inhibitor which poisons the RNA pol. II via the Elongator complex (Frohloff et al. 2001; Jablonowski et al. 2001b; Li et al. 2001). The zymocin resistant phenotype of *kti14* mutants is dependant on the C-terminal region integrity of the protein (Fichtner et al. 2002b), further more the expression of Urm1 and Uba4 in *elp* mutants (Fichtner et al. 2003). All these proteins were involved in wobble uridine nucleoside modification at position 34 of certain tRNA molecules (see below).

On gene expression, almost 100 genes were regulated in *elp* mutants, and the profile of up- or down-regulation of the target genes was very similar (Krogan and Greenblatt 2001). Chromatin immunoprecipitation (ChIP) and Re-ChIP assay suggested that the Elongator complex could bind to the elongating transcript after the RNA pol. II is hyper-phosphorylated (Métivier et al. 2003). In other words, the Elongator complex starts its mission after the commitment of RNA pol. II. Recent study with ChIP assay also showed that the Elongator complex could associate with genomic DNA regions within repilons and DNA replication-coupled histone acetylation (Xu et al. 2012). Since genes in different cellular pathways were affected in *elp* mutants, pleiotropic phenotype was illustrated in higher eukaryotes during the process of embryogenesis and organ development.

### Pleiotropic phenotype in higher plants

Mutation in both structural and regulatory components of the Elongator complex in *A. thaliana* results in multiple phenotypes including narrow leaves, enlargement of hypocotyl, retarded primary root growth, decreased seed germination, delay of flowering time, and reduced apical dominance (Nelissen 2003, 2005; Chen et al. 2006; Zhou et al. 2009). The growth phenotypes were associated with decreased cell division rate, defect in control of cell cycle and meristem polarity setup (Xu et al. 2012). Transverse section of *elp* and *kti12* mutants showed fewer and larger palisade cells (Nelissen 2003, 2005). By transmission electron microscopy, Falcone et al. (2007) observed that the *atelp4* mutant had fewer stacked-grana in chloroplast, a hypotonic vacuole and massive exocytosis; and the same mutant displayed different growth dynamics and gene expressions in response to sucrose. The apical dominance phenotype was attributed to misregulation in biosynthesis or transport of plant hormones including anthocyanin, abiscic...
acid, auxin, and abscisic acid (ABA) (Nelissen 2005, 2010; Zhou et al. 2009). Auxin biosynthesis, transport, perception, and signalling genes were severely affected according to the microarray data from elp mutants (Nelissen et al. 2010), gene ontology (GO) clustering also revealed significant change for genes in chromatin assembly, pattern specification and vascular tissue development. Free indoleacetic acid (IAA), ethylene, jasmonic acid (JA) and anthocyanin content in mutants of ELP1, ELP2, ELP4, and ELP6 were higher than that in wild type, which might involve regulation by transcriptional factors (Zhou et al. 2009) and epigenetic modification on genomic sequence by H3K14 (Nelissen et al. 2010). Leaf abaxial/adaxial polarity was altered in Arabidopsis elp mutants, possibly due to decreased cell division and DNA replication coupled with regulation of chromosome structure by histone modification (Xu et al. 2012). The crosstalk between salicylic acid (SA), JA/ethylene (ET) and ABA also partially explained the pleiotropic phenotypes of elp mutants in higher plants (DeFraia and Mou 2011), along with secondary effect of hormone imbalance on gene expression involved in abiotic stress, pathogen response and anthocyanin biosynthesis (Nelissen et al. 2010).

Drought and other abiotic stress can cause similar changes in cellular pathways as oxidative stress. Surprisingly several Arabidopsis elp mutants were found to be more tolerant to oxidative reagent methyl-viologen and H2O2 (Chen et al. 2006; Zhou et al. 2009), further more elp1 mutant was more drought tolerant than wild type (Chen et al. 2006). Leaf stomata closure and root elongation of elp1 and elp2 mutants were more sensitive to plant hormone ABA (Chen et al. 2006; Zhou et al. 2009). It is noteworthy that elp4 and elp6 mutants did not have stomata closure and water retention phenotype as that in elp1 and elp2 mutants (Zhou et al. 2009; Nelissen et al. 2010), suggesting functional difference between the Elongator complex core-subunit and sub-complex. On gene expression, both ABA responsive genes and several drought-stress related genes were less induced in elp mutants than in wild type (Chen et al. 2006), suggesting the elevated tolerance for drought and oxidative stress might not be transcriptional but translational instead. In another study, two transcriptional factors and three genes involved in oxidative and abiotic stress were differently expressed in Arabidopsis elp mutants, either with or without ABA treatment (Zhou et al. 2009). Therefore it is still on debate whether regulation of gene expression by the Elongator complex is mostly transcriptional or translational.

By constructing double mutants of the KTI12 and various ELP genes and comparing the phenotype of double mutant (DM) with their parents, epistatic relationships between the Elongator complex subunits and regulatory components were determined as the following: 1) KTI12 is epistatic over ELP genes; 2) subcomplex gene ELP4 is epistatic over core complex genes ELP1-3; 3) within core complex ELP1 is epistatic over ELP3 (Nelissen et al. 2010). Considering the hetero-hexameric ring-like structure of the Elp4-6 subcomplex with the Elp1-3 core-complex (Lin et al. 2012), in addition with the regulatory components either with or without direct physical interaction with the Elongator complex proteins, these data suggests the significant role of regulatory proteins and the importance of Elp1 as scaffold protein for the proper function of the whole Elongator complex. The Elongator complex proteins were found to be located mainly in cytoplasm except for Elp3 (Nelissen et al. 2010), and the tissue specific expression patterns of ELP2, ELP4 and ELP6 genes were very similar to that of ELP1 in Arabidopsis (Chen et al. 2006; Zhou et al. 2009). Co-localization of Arabidopsis Elp3 protein with euchromatin was verified, acetylation level of histone H3K14 on auxin-related genes was found to be reduced which resulted in down-regulation of the target genes (Nelissen et al. 2010). Because H3K14 is the predominant substrate of Elp3 HAT activity, the epigenetic regulation of auxin biogenesis and transport genes partially explained the auxin-biology-related phenotypes of Arabidopsis elp mutants such as reduced apical dominance, changed phyllotaxy, defective leave venation patterning, and reduced root growth (Nelissen et al. 2010).

Besides HAT activity, Elp3 protein also contains a radical shoot apical meristem (SAM) domain, which has been shown recently in mice for paternal DNA demethylation (Okada et al. 2010). As the Elongator complex works as a whole functional unit, knock out of other structural genes also lead to similar results on DNA methylation status. Recent study suggests role of ELP2 in plant immune response towards certain pathogenic bacteria (DeFraia et al. 2010). Arabidopsis elp2 mutant
showed much less of free SA upon pathogen attack, because SA is important for defense-related signal transduction in plants. Elp2 is considered as an accelerator of defense response. Although not required for systematic acquired resistance, AtELP2 regulates both SA accumulation and kinetics of defense gene induction, therefore it is considered as a component for basal immunity (Defraia et al. 2010). elp2 mutant in Arabidopsis also influenced pathogen-induced DNA methylation/demethylation on at least two defense genes at specific sites, and histone H3 acetylation levels in several defense genes were also compromised (Wang et al. 2013). Both increased DNA methylation and decreased H3 acetylation contribute to the delayed defense gene induction, this epigenetic regulation both in zygotic cell and somatic cell adds an additional level of gene regulation by the Elongator complex. However the epistatic relationship between histone acetylation and DNA methylation/demethylation need further investigation.

Association with embryogenesis and human disease

Human familial dysautonomia (FD) is a neurodegenerative disease, IKAP/hElp1 protein level was found to be very low in brain tissues from FD patients (Anderson et al. 2001). Elp3 protein is unstable without association with Elp1, therefore Elp3 level is also reduced in FD patients (Close et al. 2006). Cells with low levels of the Elongator complex display defects in cell motility and migration, it has been shown that Elp3 promotes α-tubulin acetylation and also physically interacts with it (Creppe et al. 2009). Elp1 and Elp3-mediated tubulin acetylation is found in C. elegans (Chen 2009; Solinger 2010), human and mouse (Creppe et al. 2009), which was manifested by defects in early embryogenesis or neurological dysfunction. Since several neurodegenerative disease including Alzheimer’s, Parkinson and ALS have been linked to defects in intracellular trafficking, the loss of elongator-mediated tubulin acetylation can lead to defective intracellular cargo transport therefore survival of neuronal cells (Defraia and Mou 2011). Alternatively, dysfunction of the Elongator complex also results in general translation defect which is more sensitive for neural cell development. Elp3 dysfunction causes embryo-lethality in D. melanogaster, microarray analysis suggested considerable overlap of gene expression involved in neuronal development with domino mutant (Walker et al. 2011).

THE ELONGATOR COMPLEX IN ASSOCIATION WITH tRNA WOBBLE URIDINE MODIFICATIONS

Yeast mutants of ELP1-6 and KTI11-14 all lack ncmU (5-carboxomethyluridine) and mcmU(l)U (methoxy-carbomethyl-(thio-)uridine) at position 34 (wobble position) in total tRNA (Huang 2005, 2008). All of these mutants displayed zymocin resistance phenotype in S. cerevisiae. Study by Lu et al. (2005) revealed that modified uridine is the recognition site of the zymocin tRNA endonuclease in vitro, therefore mutants lacking these wobble uridine modification were resistant to zymocin. In S. cerevisiae 11 tRNA species carried either ncmU or mcmU derivatives at position 34 (Johansson et al. 2008), overexpression of hypomodified tRNA\textsuperscript{Gln}_{ncmU} and tRNA\textsuperscript{Glu} suppressed growth phenotypes as well as exocytosis defects in elp mutants (Esberg et al. 2006) and partial resistance towards zymocin, suggesting that the phenotype is mainly due to inefficient translation. Overexpression of three tRNA species containing hypomodified mcm\textsuperscript{s}U also suppressed the defects in telomeric gene silencing and DNA damage response in C. elegans elp mutants (Chen et al. 2011), therefore at least two cases have suggested translational defects might be the primary reason for the various phenotypes observed in elp and kti mutants. According to sequenced tRNA data from Modomics database (http://modomics.genesilico.pl/sequences/list/tRNA/), higher plants also contain mcm\textsuperscript{s}U and mnm\textsuperscript{s}U wobble uridine modifications, for example in tRNA-Gln-UUG or tRNA-Glu-UUC species from Hordeum vulgare and Triticum aestivum, respectively. Since the genome sequence of A. thaliana is known, the corresponding tRNA coding genes could be identified, and most likely these tRNAs also contain similar wobble uridine modifications. It would be very interesting to see if overexpression of hypomodified tRNA-Gln-!UG (!UC represents the anticodon, ! stands for cmmnU) or tRNA-Glu-SPC (S stands for mnm\textsuperscript{s}U and P stands for pseudouridine) could rescue some of the phenotypes in Arabidopsis elp mutants.
The function of the Elongator complex is found to be conserved from yeast to higher plant, not only because the Arabidopsis Elp1 and Elp3 protein can both substitute for protein-protein interaction and complement yeast mutant for zymocin resistance, but also that elp1, kti12 and elp3 knock-out mutants lacks ncm^{3}U and mcm^{3}U modified nucleoside in total tRNA (Chen et al. 2010; Mehlgarten et al. 2010). elp1 and elp3 mutants of C. elegans also resulted in deficiency for ncm^{3}U and mcm^{3}U nucleoside modifications in total tRNA (Chen et al. 2009), supporting the view that the function of the Elongator complex in tRNA wobble uridine modification is conserved.

The codon bias in mRNA results in regulation of specific protein level mediated by the presence of modified nucleoside, Cdr2 which is a central regulator for mitosis and cytokinesis is under such translational control by Elp3 due to the lysine codons that are decoded by mcm^{3}S^{2}U-containing tRNAs (Bauer et al. 2012). Similarly, yeast exocytosis was affected in elp1 mutant in a process independent of the transcriptional elongation (Rahl et al. 2005). However, as more substrates were found for the HAT activity of the Elongator complex, and with radical SAM activity recently discovered, both tRNA nucleoside modification, DNA and proteins could be affected by the Elongator complex. The phenotype caused by lack of ncm^{3}U and/or mcm^{3}U modifications on tRNA is a translational effect; however the central role of the Elongator complex in association with RNA pol. II and histone modifications is transcriptional. Why mutation in ELP and KTI genes all lead to defect in wobble uridine modification is not understood, at least the involvement of ATP/GTPase activity was suggested for energy supply of the modification reaction.

Besides Elp1-6 and Kti1-14, Trm9 (Kalhor and Clarke 2003) and ALKBH8 (Leihne et al. 2011) proteins were further identified for the mcm^{3}U and mcm^{3}U modifications. It is suggested that Trm9 methylation facilitates the recognition of target tRNA by zymocin (Jablowski et al. 2006), the defect in translation also renders the mutant sensitive to paramycin at high temperature (Kalhor and Clarke 2003). However no obvious phenotype was observed under normal growth conditions for TRM9 and ALKBH8 mutants in Arabidopsis, which lacks mcm^{3}U and mcm^{3}U modified nucleosides, respectively (Leihne et al. 2011).

Acetylation of histone by the Elongator complex also need the expression of Sit4 together with its associated protein Sap185 and Sap190 (Jablowski et al. 2004), moreover yeast sit4 mutant and sap185, sap190 double mutant resembled phenotypes in lack of ncm^{3}U, mcm^{3}U and mcm^{3}S^{2}U modified nucleoside and zymocin resistance (Huang et al. 2008). The phosphorylation status of Elp1 was suggested to be regulated by Sit4 phosphatase with the help of Sap185/Sap190, which is antagonized by Kti12 and Kti14 (Mehlgarten et al. 2009).

**OTHER ROLES OF THE ELONGATOR COMPLEX**

The Elongator complex has also been implicated to be involved in cytoplasmic kinase signalling and yeast exocytosis, however, there is some controversy with the results from later study, therefore there is still debate concerning whether the Elongator complex is participating in these pathways directly or indirectly (Svejstrup 2007).

The human Elp1 was firstly identified by association with IxB kanases, however this was shown with overexpression of the Elp1 protein (Cohen et al. 1998). Later study basically repudiated this conclusion and suggested that Elp1/IKAP had no specificity in cytoplasmic kinase signalling (Krappmann et al. 2000). The interaction of Elp1 with JNK kinase by yeast-two-hybrid was also performed with over-expression approach (Holmberg et al. 2002), indeed numerous studies failed to support any involvement of Elp1 or other subunits of the Elongator complex in kinase signalling (Svejstrup 2007).

Using a yeast sec2-52 mutant which harbours a premature stop-codon in the essential Rab GTPase, Rahl et al. (2005) illustrated the possible role of the Elongator complex in exocytosis. Since the author used a Elp1 construct with strong nuclear-localization-signal, the results might be misleading and could not represent the actual function of the Elongator complex in exocytosis. However, Esberg et al. (2006) found that the exocytosis defect of yeast sec2-59 mutant could be overcome by elevated level of hypomodified tRNA-Lys and tRNA-Gln which normally contain mcm^{3}S^{2}U at wobble position, suggesting the phenotype is due to a translational defect. The increase levels of hypomodified tRNA-Lys and tRNA-Gln in the elp1 mutant strain could restore the po-
larized localization of Sec2 protein which is essential for exocytosis, suggesting the Elongator complex’s function in exocytosis is connected with its role in tRNA wobble uridine modification (Esberg et al. 2006).

CONCLUSION

The Elongator complex is an important functional unit for transcriptional elongation (Lu et al. 2007; Svejstrup et al. 2007; Versees et al. 2010; Creppe et al. 2011). Elp3 is both in a core structural subunit and the catalytic subunit for the whole complex, which is involved in acetylation of H3 and H4 histone lysine residue as well as other substrates including tubulin (Winkler et al. 2002; Creppe et al. 2009; Solinger et al. 2010). Recently an Elp3 protein from insect Nilaparvata lugens was indentified, the phylogeny of NiElp3 with homologs of the GNAT superfamily from other organisms was illustrated (Zhu et al. 2013). The NiElp3 protein also contains radical SAM domain, possibly also involved in DNA demethylation. It has been shown that affinity binding to DNA and histone substrates need the presence of whole complex (Winkler et al. 2002).

Up to date the multifunctional Elongator complex has been shown to be involved in cell cycle control, DNA demethylation and DNA damage repair, regulator of plant abiotic stress and immune response, tubulin acetylation and cytokinesis, neuron development and human neuron degenerative diseases. Elongator-mediated telomeric gene silencing was mainly a translational effect due to defect in mcm5’s2U modification of certain tRNA (Chen et al. 2011), however, association of the Elongator complex with DNA replication, gene silencing and DNA damage response is restricted to the nucleus (Li et al. 2009). The misregulation of cell cycle and mitosis in elp mutants led to pleiotropic phenotypes in higher plants, such as elongated leaf and petioles, aberrant lateral shoot growth, delayed root growth, and meristem polarity (Xu et al. 2012). Elongtor’s association with the proliferating cell nuclear antigen (PCNA) was illustrated both in budding yeast and Arabidopsis, and this interaction was shown to be necessary for efficient histone acetylation coupled with DNA replication (Li et al. 2009; Xu et al. 2012).

The crystal structure of Elp4-6 subcomplex was identified recently (Lin et al. 2012), however the structure of the holo-elongator complex and the Elp1-3 core subunits still hinders the understanding of the involvement of the Elongator complex in different biochemical pathways. A schematic view of the Elongator complex is represented here based on the present knowledge of the structure and biological functions within eukaryotes (Fig.). The subcellular localization of each subunits and their regulation for protein and gene expression under transcriptional or translational control, in particular the link between dysfunction of particular tRNA modification and downstream targets and phenotype manifestation is a challenging work for the future.

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