

Elongator

Transcriptional or translational regulator?

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The conserved multisubunit Elongator complex was initially described as an RNA polymerase II (RNAP II) associated transcription elongation factor but, since, has been shown to be involved in a variety of different cellular activities. Here, we summarize recent developments in the field and discuss the resulting implications for the proposed multi-functionality of Elongator.

Introduction

Elongator is a large macromolecular complex (~900 kDa) that is built up by two copies of each of its six subunits (named Elongator proteins Elp1–6).¹ The importance of each of the individual subunits for Elongator function is not only highlighted by the high degree of sequence conservation from yeast to human, but also by the fact that the deletion of any of the subunits leads to the loss of Elongator complex integrity, its dysfunction and almost identical phenotypes in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis thaliana* and *Drosophila melanogaster*.²⁻⁷ Since its discovery, the cellular functions of Elongator are controversially discussed,^{8,9} as it has been implicated in different cellular activities, including protein acetylation,¹⁰⁻¹² exocytosis,¹³ sensitivity to DNA damaging agents,¹⁴ zygotic paternal DNA demethylation¹⁵ and tRNA modification¹⁶ (summarized in **Fig. 1**).

In humans, several studies have shown that patients suffering from certain neurodegenerative diseases carry mutations or variants in one of the six human Elongator

genes.¹⁷⁻¹⁹ A complete mechanistic understanding of specific roles of Elongator in nerve cells is still missing, but it has been suggested that nerve cells are particularly sensitive to translational defects because of their markedly higher rates of protein synthesis compared with other cell types.^{5,12,20} Contradictory reports present Elongator as a dual regulator of transcription and translation complicating the quest for a unique targeted treatment strategy for the benefit of the patients. Clarifying whether Elongator regulates a multitude of cellular activities through a global transcriptional or translational mechanism therefore has become a high priority.

Elongator—A Transcriptional Regulator?

The presence of a conserved histone acetyl transferase (HAT) domain in Elp3¹¹ and co-purification of Elongator with RNAP II²¹ initially led to the conclusion that Elongator might facilitate transcription elongation by acetylating histone tails, a post-transcriptional chromatin mark generally associated with actively transcribed genes.²² Surprisingly, a genome wide study on transcription elongation factors could not find any association or enrichment of Elongator at actively transcribed genomic regions.²³ Later, Elongator was believed to interact with nascent mRNA during transcription elongation,²⁴ but in a recent study, designed to identify the complete human interactome of chromatin-associated messenger ribonucleoproteins by proteomics, no Elongator subunits

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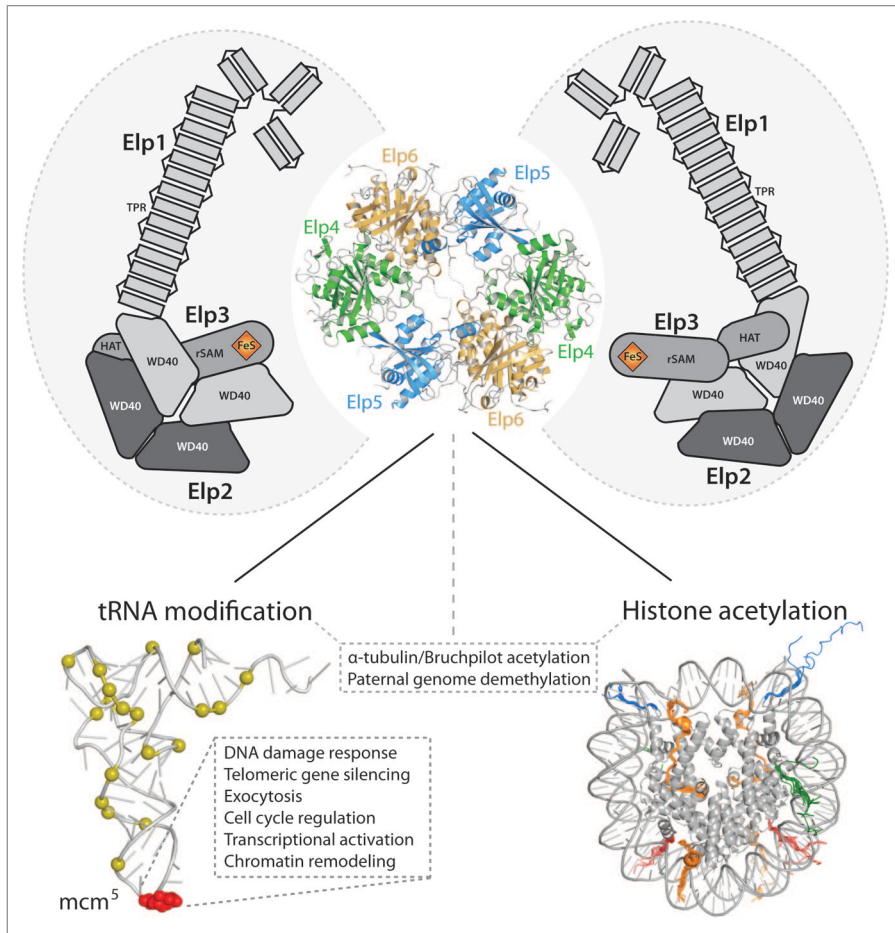


Figure 1. Elongator complex assembly and its proposed cellular functions. The upper panel shows a schematic representation of the Elongator subcomplex arrangement. The central hexameric Elp456 subcomplex is shown in cartoon representation (Elp4 in green; Elp5 in blue; Elp6 in brown) and is flanked by two copies of the Elp123 subcomplex. Predicted domains in Elp123 are marked individually [WD40 domains, tetratricopeptide repeats (TPR); histone acetyl transferase (HAT); radical S-adenosyl-methionine (rSAM); iron-sulfur cluster (FeS)]. The lower panel highlights tRNA modification (translational control) and histone acetylation (transcriptional control) as Elongator's proposed cellular functions. Additional functions, which were shown to be indirectly affected by Elongator role in tRNA modifications are DNA damage response,⁴⁴ telomeric gene silencing,⁴⁴ exocytosis,¹⁶ cell cycle regulation,³⁵ transcriptional activation¹⁶ and chromatin remodeling.¹⁶ Functions that could indirectly result from translational/transcriptional activities of Elongator or because the proteins involved are additional direct targets of Elongator such as α -tubulin/Bruchpilot acetylation¹² or paternal genome demethylation¹⁵ are listed separately.

could be detected, either.²⁵ Furthermore, the sub-cellular localization of Elongator subunits seems to be mainly cytoplasmic. This localization apparently contradicts its assigned role in transcriptional elongation, although it was shown that some subunits in certain organisms could localize to the nucleus.^{10,12,26-29} Although Elp3 was indeed reported to acetylate histones *in vitro*,^{11,27} it was recently implicated in the acetylation of Bruchpilot, an integral component of the presynaptic density at the periphery of neuronal cells.¹⁰ This modification

could directly link Elongator-dependent protein acetylation to the reported neuronal phenotypes of patients with Elongator mutations.

In summary, although the observation that Elongator is involved in a whole variety of cellular activities could indeed be explained by its ability to impact on the transcription of a set of genes that are involved in those cellular activities, there is accumulating evidence in the literature that the initial concepts suggesting a direct

involvement of Elongator in transcription elongation by RNAP II have to be confirmed and challenged by future *in vivo* and *in vitro* studies.

Elongator—A Translation Regulator?

Elongator was shown to directly interact with eukaryotic tRNAs *in vitro*^{1,3} (but not with single stranded DNA or RNA) and is required for the formation of 5-methoxycarbonylmethyl (*mcm*⁵) and 5-carbamoylmethyl (*ncm*⁵) groups on uridine nucleosides present at the wobble position of many tRNAs.^{3,30} During a screen for yeast factors conferring resistance to the *Kluyveromyces lactis* γ -toxin (zymocin), all six Elongator genes were identified together with other factors known to affect the tRNA modification of the same tRNA residue.³⁰ Consistent with the observation that Elongator mutant strains are resistant to zymocin, it was shown that *mcm*⁵ modified uridines in tRNAs represent the specific target sites for the toxin.^{2,31} Strikingly, Esberg and colleagues¹⁶ have shown that most cellular phenotypes, including those originally associated with the role of Elongator in transcription elongation, could be rescued by overexpressing two yeast tRNAs whose modifications are Elongator-dependent. This thereby provided the first body of evidence that the function of Elongator in tRNA modification might result in a wide variety of phenotypes through its global impact on translation (Fig. 1).

The biochemical details of the contribution of Elongator (together with other factors) to tRNA modification are still unresolved, although it was recently shown that so-called radical S-Adenosyl Methionine (SAM) domains, of which one is present in the N-terminus of Elp3,³² are able to mediate the transfer of methyl groups to RNAs.³³ Strikingly, mutations affecting the conserved active site residues in the HAT domain of Elp3, formerly exclusively associated with histone acetylation, result in the loss of tRNA modification activity of Elongator.³ The formation of the rather complicated *ncm*⁵/*mcm*⁵ modification, which occurs, for a few tRNAs, on the same wobble uridine in the wobble base position that is also

thiolated, enhances translation efficiency and accuracy.³⁴ A very recent report by Bauer and colleagues clearly supports that Elongator is indeed affecting the translation of a whole variety of cellular proteins.³⁵ Strikingly, these translational alterations are tightly correlated with the codon usage of the affected genes, namely the bias toward codons, that are translated by Elongator-dependent modified tRNAs.

In summary, the surprising finding that certain tRNA species can rescue phenotypes originally associated with Elongator's direct role in several cellular activities, might be simply explained by its tRNA modification activity and the subsequent changes in the translation of a whole variety of target mRNAs.

Structural Dissection of Elongator and the Consequences for its Substrate Recognition

Recently, the first structural information of a part of the Elongator complex at atomic resolution was obtained, namely the crystal structure of the Elp456 subcomplex.^{1,36} The crystal structure, in combination with additional structural, biochemical, biophysical and *in vivo* analyses, revealed the hetero-hexameric nature of the Elp456 subcomplex (Elp456). Unexpectedly, Elp4, Elp5 and Elp6 all share the same RecA like protein fold and are arranged in a ring-like fashion, known from homo-hexameric members of the RecA-like NTPase family.³⁷ The hetero-hexameric assembly provides a logical explanation to the earlier observation that single deletions of the three Elp456 subunits lead to identical *in vivo* phenotypes. In addition, it was shown that the hexameric conformation of Elp456 is essential for its ability to bind to the anti-codon stem-loops (ACLs) of tRNAs.¹ tRNA binding was shown to be abolished in mutants targeting the central cavity of the ring, particularly the homologous nucleic acid binding loops (L2) of Elp6, known from RecA, Rho, and other members of the hexameric NTPases to bind specifically to nucleic acids. Similar to other RecA-like NTPases, Elp456 is able to hydrolyze ATP and to use this hydrolysis reaction to regulate its association to tRNAs.¹ In this scenario, ATP recruitment and hydrolysis

in the Elp456 ATPase sites, located on the outside of the inter-subunit interfaces, would induce conformational changes in the distant tRNA binding loops, located in the central cavity, allowing the release of a tRNA molecule. After ATP hydrolysis, the hydrolyzed nucleotides would be released, which would allow Elp456 to bind to the next tRNA molecule and induce its modification. How the temporal control of this regulatory circuit is orchestrated is not fully understood yet, but intensive research efforts are currently ongoing.

The conservation throughout evolution of a hetero-hexameric ring might be simply explained by preserving substrate specificity toward tRNA stem loops or other particles harboring pseudo 2-fold symmetry. In addition, a hetero-hexameric assembly facilitates the specific positioning of only two copies of the associated Elp123 subcomplexes on a hexameric central Elp456 particle. It was shown that the above described structure-function analysis of the separated Elp456 hexamer can be translated into the fully assembled Elongator complex, as Elp4, Elp5 and Elp6 are incorporated into the full complex as a hexameric assembly.

Discussion and Conclusions

Obviously, the most intriguing questions concern the substrate specificity and enzymatic activity of the Elongator complex. Although data on its involvement in tRNA modification are recently accumulating,^{1,3,16,35} further supporting a role of Elongator in this activity, the existence of additional substrates and molecular activities more related to its role in transcription regulation cannot be fully excluded yet. Some Elongator functions might also only be found in certain organisms and thereby underline the active evolutionary process that has affected this macromolecular complex. The possibility remains that alternative assemblies of Elongator subunits exist in the cell that catalyze different molecular activities. Indeed, if it was reported that Elongator dissociates into two subcomplexes at higher salt concentration,³⁸ it remains possible, for example, that individual subunits might interact with different associated factors.^{29,39-43}

The latter would, however, be inconsistent with the similar phenotypes observed upon inactivation of different Elp subunits. Detailed co-localization studies of all six Elongator proteins in different organisms should help clarify if alternative compositions exist, are specific to certain organisms, and are localized in different sub-cellular compartments.

Structural analyses of subunits Elp1, Elp2, Elp3 and the fully assembled Elongator complex will allow a better molecular understanding of this large complex in the future. On one hand, low and medium resolution analyses of the fully assembled Elongator will help understand inter-subunit communication and the influence of associated factors and substrate recognition for the overall architecture of Elongator. On the other hand, high resolution structural information will allow a detailed description of the chemical reactions catalyzed by Elongator during tRNA modification, interaction with partners involved in this process and, potentially, other modifying activities of the Elongator complex. Further investigations of the tRNA recognition mechanism by Elongator might also lead to a structural explanation of how the anti-codon loop is recognized and why only uridines in the wobble position of the tRNA are subject to this specific modification reaction, whereas uridines in other positions remain unmodified. In summary, novel structural insights will continue to complement known and ongoing functional studies on Elongator and will contribute to understand the true nature of this highly conserved cellular machine.

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