The Elongator complex (Elp1-Elp6) was originally identified as a component of hyperphosphorylated RNA polymerase II (RNAPII) 2 holoenzyme isolated from budding yeast chromatin (1). The Elp3 subunit of Elongator harbors motifs found in the Gcn5-related N-acetyltransferase family of histone acetyltransferases (HATs) (2, 3). Indeed both yeast and human Elongator have HAT activity in vitro primarily directed toward histone H3 (4–6), and deletion of yeast ELp3 results in decreased histone H3 acetylation levels in chromatin in vivo (4, 7). Depletion of human Elongator complex from HeLa nuclear extracts decreases acetyl-CoA-dependent transcription through a chromatin template that can be restored by adding back purified Elongator complex (6). In further support of a role in transcription, RNA immunoprecipitation revealed that Elongator is associated with the nascent RNA emanating from elongating RNAPII along the coding region of several yeast genes (8), and chromatin immunoprecipitation experiments from several laboratories demonstrated an association of Elongator with active genes in human cells (9–11). Moreover RNA interference-mediated depletion of the 1Kb kinase complex-associated protein/ hElp1 subunit of Elongator in human cells results in transcription defects at numerous genes. These genes exhibit reduced histone H3 acetylation and decreased RNAPII density specifically toward their 3′-end (10).

Interestingly the majority of Elongator is cytoplasmic in most cell types investigated (6, 12), 3 indicating that the complex performs additional distinct functions in the cell. In yeast, genetic data have provided strong evidence for a role for Elongator complex in tRNA modification (13, 14). However, the precise cytoplasmic role of Elongator is unclear, and the relationship, if any, between its nuclear and cytoplasmic functions also remains poorly understood (for a review, see Ref. 15).

Genetic screening in yeast for mutations that confer resistance toward the otherwise lethal intracellular expression of the killer toxin (zymocin) γ subunit identified genes that were named TOTI–7 (toxin target) (16–18). These genes either encode subunits of yeast Elongator or the Kti12 protein. ELp1 and KTI12 had been isolated previously in independent screens for mutants that render cells resistant to the native toxin (insensitive to killer (IK) and killer toxin-insensitive (KTI) genes, respectively) (19, 20). These screens also identified the Kti11 protein. Neither Kti11 nor Kti12 is stably associated with Elongator, but both have been shown to physically and functionally interact with the complex (17, 21, 22) and are also required for tRNA modification (14). Unlike Elongator, which has proved recalcitrant to detection at genes in yeast by chromatin immunoprecipitation, Kti12 is readily detected on genes by this assay (21), suggesting that it may also affect Elongator function in the nucleus. Thus, further investigation of the relationship among Kti11, Kti12, and Elongator is warranted.

The study reported here was initiated in response to the finding, by sequence alignment, that the Elp3 protein potentially harbors an iron-sulfur cluster (FeS) domain, which was hypothesized to function catalytically in histone demethylation (23). A
subsequent study revealed that archael Elp3 indeed has an unstable FeS cluster (24), making it even more pressing to know the importance, if any, of this domain for Elp3 and Elongator function in eukaryotes. Here we provide evidence that the Elp3 FeS cluster is functional and crucial for yeast Elongator but that it does not appear to be involved in the known catalytic role(s) of the complex. Rather our evidence supports the idea that it provides structural stability and enables interaction with Kti11 and Kti12. In contrast, Elongator interactions with RNAPII in chromatin do not appear to require this motif.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**—All of the Saccharomyces cerevisiae strains used for genetic analyses were congenic with strain W303 (leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-1) and were grown and manipulated using standard techniques. The elp3Δ strain has been described previously (2) as has the strain expressing C-terminal His10-hemagglutinin (HisHA)-tagged Elp1 (25). Details of genomic tagging of the C terminus of Elp1 with His6-TEV2-Myc18 (MycHis) are available on request.

A plasmid containing the ELP3 coding sequence with upstream and downstream regulatory sequences was made previously (3). Point mutations in the putative iron-sulfur cluster were generated using a QuikChange XL site-directed mutagenesis kit (Stratagene) and confirmed by sequencing. Methods for construction of the Y540A mutant as well as for growth of elp3Δ strains expressing different Elp3 mutants were described previously (3).

**Western Blotting and Antibodies**—Western blotting was carried out using standard techniques. Elp3, Elp4, and Elp5 antibodies were described previously (2, 25).

**Purification of Wild Type and Point-mutated Elongator Complexes**—Elongator expressing Elp1 tagged at the C terminus with HisHA was purified as described previously (25). To purify complexes from the soluble fraction of the MycHis-tagged strains, cells were grown to a density of ~1.5 × 10^7 cells/ml, harvested by centrifugation, washed in cold water, and flash frozen in small droplets in liquid nitrogen, and stored at -80 °C. Details of purification under anoxic conditions are described in the supplemental material.

**HAT Assays**—HAT assays were carried out in a 40-μl reaction volume consisting of 10 μg of core histones (Upstate), 6 μM [3H]acetyl-CoA (2–10 Ci/mmol, Amersham Biosciences), and 10–20 μl of purified Elongator in HAT buffer (40 mM HEPES-KOH, 5 mM Na butyrate, 5 mM MgCl2, 25 mM NaCl, 100 mM potassium acetate, 5 mM dithiothreitol, pH 7.5). Following a 30-min incubation at 30 °C, reactions were stopped by the addition of 14 μl of SDS-PAGE loading buffer and fractionated on 18% polyacrylamide gels. After Coomassie staining, gels were soaked in Amplify solution (Amersham Biosciences) for 30 min and dried, and histone bands were detected by fluorography.

**In Vitro and in Vivo RNA Binding Assays**—RNA electrophoretic mobility shift assays and RNA immunoprecipitations were carried out as described previously (8).

**RESULTS**

**A Functional Iron-Sulfur Cluster in the Yeast Elp3 Protein**—The Elp3 subunit of Elongator has a HAT domain located at its C terminus (2, 4). More recently, the existence of a second conserved N-terminal domain was proposed based on sequence alignment (23). This domain bears homology to the catalytic domain of radical S-adenosylmethionine (AdoMet) enzymes and contains a cysteine-rich motif that might constitute an FeS cluster (Fig. 1A). Indeed the involvement of this motif in the formation of such a cluster in Methanococcus jannaschii Elp3 was recently supported experimentally (24).

Experiments to study the biophysical characteristics of the FeS cluster in the context of Elongator could not be pursued because of the large quantity of protein (mg amounts) required. We therefore initially set out to investigate the role of this motif in regard to Elongator function in vivo. To this end, point mutations (to alanine) were made at positions Cys-103 (a non-conserved cysteine bordering the conserved domain) and the highly conserved Cys-108, Cys-118, and Cys-121 residues in the
yeast Elp3 FeS cluster (Fig. 1A). To examine the phenotypic consequences of these point mutations, CEN plasmids expressing the mutant versions of ELPS3 from its own promoter were generated and transformed into an elp3Δ strain. In addition, plasmids expressing wild type (WT) Elp3 or no Elp3 were included as positive and negative controls, respectively. WT Elp3 and all mutants were expressed at similar levels as determined by Western blotting, and the ELPS3 mutations did not affect the levels of another Elongator component, Elp5 (Fig. 1B). The results of an experiment examining temperature and salt sensitivity, both of which affect the growth of cells lacking ELPS3 (2), are shown in Fig. 1C. Only cells expressing Elp3 mutated at the non-conserved Cys-103 position were able to grow normally at 37 °C and in the presence of 1 M salt. All other point mutants displayed growth indistinguishable from that of the elp3Δ strain. elp3Δ-like behavior was also observed for the same cysteine mutants in other phenotypic assays for Elongator function, such as synthetic slow growth with gcn5 mutation (3) and insensitivity to killer toxin (26) (supplemental Fig. S1). These results demonstrate that single point mutations in the conserved N-terminal FeS cluster of Elp3 are sufficient to cause an elp3Δ growth phenotype, indicating that this motif is crucial for Elongator function.

Purification of WT and Point-mutated Elongator—To investigate the effect of the Elp3 mutations on the activity of Elongator in vitro, we attempted to purify point-mutated Elongator to homogeneity. Previously a two-step protocol for purification of the WT complex via a HisHA tag at the C terminus of the Elp1 subunit was described (25). After tagging Elp1 with HisHA in elp3Δ cells expressing WT Elp3 from a CEN plasmid, we used the phenotypic assays described above to confirm that the cells had normal Elongator function (data not shown). As expected, expression of the Elp3 C108A point mutant in place of WT gave rise to an elp3Δ phenotype (data not shown). Elongator was then purified from 100-liter cultures of these strains as outlined in Fig. 2A (left panel), and the ensuing protein fractions were analyzed by SDS-PAGE (right panel). Purification from cells expressing the C108A mutant complex was unsuccessful with Elp1 and contaminants comprising the majority of the resultant protein. Even upon extended silver staining, bands representing Elp2 and Elp3 failed to become visible (Fig. 2A, right panel (upper)). Moreover little or no Elp3 was detected by Western blotting (Fig. 2A, right panel (lower)). Bands corresponding to Elp4, Elp5, or Elp6 (comprising the smaller Elongator subcomplex) also failed to appear upon silver staining of the mutant complex (Fig. 2A, right panel (upper)), although some partly degraded Elp5 protein was detected by Western blot (Fig. 2A, right lower panel (lower)). The proteins contaminating the purifications seemed to differ between successive preparations and were not pursued further.

It was evident from these and several other similar experiments that it was not possible to purify the point mutants using the procedure developed previously for WT Elongator (25). One possible explanation for these observations was that although Elp3 expression and stability was largely unaffected by the introduced point mutations (Fig. 1B) they might have an effect on complex assembly and/or stability. More specifically, we hypothesized that point-mutated Elp3 was either unable to form a complex with the remaining Elongator subunits or that the subunit interactions in the complex were altered, resulting in a complex that dissociated and/or became degraded during purification.

Co-immunoprecipitation of Elongator Subunits from Cells Expressing WT and Point-mutated Elp3—To address the question of whether holo-Elongator could be assembled with a point-mutated Elp3 subunit, we attempted to immunoprecipitate the complex directly from whole cell extract using the single HA affinity tag at the C terminus of Elp1. However, insufficient amounts of Elongator were precipitated using this
approach (data not shown). Therefore, CEN plasmids expressing WT or C108A Elp3 were transformed into an elp3Δ strain in which we had tagged Elp1 with 18 Myc epitopes (27). 9E10 (anti-Myc) immunoprecipitation was then performed. Fig. 2B shows the results for a strain expressing WT Elp3 and untagged ("No tag") or tagged (Elp1-Myc) Elp1, respectively. As expected, in the absence of a tag on Elp1, neither Elp3 nor Elp5 were removed from the flow-through relative to the input. Thus, under these experimental conditions, there was little or no nonspecific binding of Elongator to the 9E10 beads. In contrast, the Myc18 tag on Elp1 resulted in substantial co-depletion of Elp3 and Elp5 from the flow-through and a clear concentration of all three subunits in the immunoprecipitated material, indicating that the complex was intact. More importantly, the results for C108A Elp3 looked similar (Fig. 2C). Together these results suggest that holo-Elongator is indeed assembled in both the WT and mutant strains but that the mutant complex dissociates during the multistep purification procedure. Similar results were obtained with C118A and C121A Elongator complexes (data not shown).

Design and Use of a New Purification Protocol for Elongator—

The results presented above support the idea that point mutations in the N-terminal motif of Elp3 cause a decrease in stability of the Elongator complex. The integrity of FeS clusters is generally very sensitive to oxidation, and therefore it is not surprising that mutations predicted to disrupt this domain may affect the structural integrity of Elp3 and consequently the Elongator complex as a whole.

An important aim of our studies was to investigate the possibility that Elongator is a histone demethylase as suggested previously based on sequence homology to radical AdoMet enzymes (23). However, experiments performed with purified Elongator failed to uncover proof for such an activity. Likewise despite repeated attempts, we also failed to find any evidence for an ability of Elongator to bind AdoMet (see “Discussion”). We surmised that these failures might possibly be due to rapid inactivation of the FeS cluster (for example, by oxidation) during Elongator purification. Therefore, key aims of a new purification protocol were to increase the speed of the procedure and minimize the number of freeze-thaw cycles, the salt concentration, and the oxidative pressure.

To this end, a new Elp1 C-terminal multiple epitope tag was designed (Fig. 3A). Given the success of the 9E10 immunoprecipitation experiment wherein Elongator was both quickly and effectively isolated at low salt concentration, we again utilized a Myc18 tag. To facilitate efficient elution from the antibody resin, tandem recognition sites for TEV protease were incorporated between the Myc sequences and a His8 affinity tag at the C terminus of the protein (termed MycHis tag). The His tag was inserted to enable further purification and concentration of Elongator after TEV protease-mediated release from the 9E10 resin. Phenotypic analysis of an Elp1-MycHis/elp3Δ/elp5Δ strain expressing WT Elp3 from a CEN plasmid confirmed that the tag did not affect Elongator function or expression (data not shown).

An outline of the purification protocol developed to rapidly isolate Elongator under non-stringent conditions is shown in Fig. 3C (left panel; see “Experimental Procedures” for details).
Having optimized the purification protocol, we repeated our experiments with Elongator in the mutant cells (Fig. 3). The Western blotting (data not shown and see below) and were not able to detect significant differences in the composition of the mutant complexes. In addition, the relative intensity of the Elp2 band was reduced in the mutant complexes compared to WT. This was due to the loss of Elp3, Elp4, Elp5, and Elp6 levels (relative to Elp1) in all samples, there was a pronounced loss of Elp2 and a clear decrease in RNA binding in the mutant complex. Although the Elp1 protein could be seen in the WT strain, it was not present in the mutant complexes. This indicates that the RNA binding properties of the Elongator complex are not significantly affected by mutations in the FeS cluster motif of Elp3.

**Function of the FeS Cluster in Elp3**

To investigate the effect of the point mutations on the ability of Elongator to bind RNA in vivo, a series of RNA immunoprecipitation experiments were performed. RNA immunoprecipitation has been used previously to show the association of Elongator with GAL1 mRNA in vivo (8). This gene was therefore chosen as a convenient means to study the effect of point mutations in the FeS cluster. Elp1-MycC108A/elp3Δ cells expressing WT or mutant Elp3 were grown in galactose, and following cross-linking with formaldehyde, RNA was immunoprecipitated and quantitated as described previously (8, 30). As expected from previous results (31), there was little co-immunoprecipitation of GAL1 mRNA in the absence of Elp3 (elp3Δ) compared with a WT strain (Fig. 4C). Significantly the C108A point mutant showed only a slight decrease in RNA immunoprecipitation efficiency within the error of the experiment. As expected, the C103A mutant, which does not have an elp3Δ phenotype (Fig. 1, B and C), exhibited wild type RNA binding activity. Overall these findings correlate well with the in vitro experiments and indicate that the RNA binding properties of the Elongator complex are not significantly affected by mutations in the FeS cluster motif of Elp3.

**Purification of Elongator from Soluble and Chromatin-enriched Fractions**—Elongator was originally discovered through its association with elongating, chromatin-associated RNAPII (1). However, a more recent purification of tandem affinity purification-tagged Elongator (from the soluble, or chromatin-
free, fraction) failed to find evidence of an Elongator-RNAPII interaction (32). Other proteins have also been implicated in Elongator function, such as the chromatin-associated Kti12 protein, which can be co-immunoprecipitated with Elongator but is not a stable component of the complex (21), and Kti11, which itself contains a sulfur cluster (proposed to bind zinc) (33). Both kti11 and kti12 cells share several phenotypes with Elongator mutants (17, 18, 22). We investigated the interactions of Elongator with these factors in more detail using our new, milder purification procedure (Fig. 5). Strikingly both Kti11 and Kti12 were detected in highly purified Elongator fractions using this protocol. The proteins were not present in fractions prepared using the traditional HAHis procedure (Fig. 5A, compare lanes 1 and 2) or in a control preparation from untagged cells (compare lanes 5 and 6), showing that the interaction was specific. Perhaps more interestingly, whereas the Y540A mutation did not affect the interaction between Elongator and Kti11 and Kti12, neither of these proteins co-purified with the C108A mutant (Fig. 5A, compare lanes 3 and 4 with lane 2). This indicates that the integrity of the FeS cluster is required for the interaction of Elongator with these accessory factors.

We also purified Elongator from chromatin-enriched Elp1-MycHis extracts to test its binding to RNAPII (Fig. 5B). To again ensure that the purification of Elongator and associated proteins was specific, a control purification using chromatin from untagged control cells was performed. The largest subunit of RNAPII, Rpb1, was found to be associated with Elongator isolated from the chromatin-enriched extract (Fig. 5B, lanes 3 and 5) but not the soluble extract (Fig. 5B, lane 2). As expected, Rpb1 was absent from the control purification using the untagged strain (Fig. 5B, compare lanes 4 and 5), showing that the interaction was specific. Kti12 was found to be associated with Elongator isolated from both the chromatin-enriched and soluble extracts, but interestingly Kti11 was only found to be associated with the complex isolated from soluble extract (Fig. 5B, compare lanes 2 and 3). Moreover whereas the FeS cluster mutation disrupted binding to Kti11 and Kti12 (Fig. 5A), it did not perturb the interaction of Elongator with RNAPII in chromatin (Fig. 5C). Together these results further support the idea that Elongator has disparate functions requiring interactions with multiple different factors in different cellular compartments and that some, but not all, of these interactions are dependent on an intact FeS cluster.

**DISCUSSION**

Elucidating the precise cellular functions of Elongator is proving difficult. One outstanding question has been the role, if any, of a putative FeS cluster motif in the Elp3 subunit, which had been proposed to signify a new catalytic function, distinct from the HAT activity, such as histone demethylation (23). The

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**FIGURE 4.** HAT and RNA binding activity of wild type and C108A mutant Elongator. **A**, HAT assays with either WT (lane 1) or Elp3 C108A mutant Elongator (lanes 3 and 4). A negative control reaction lacking histones is shown in lane 2. The upper panel shows an autoradiograph of histone bands, and the lower panel shows Coomassie-stained histone bands from the same reaction. **B**, in vitro RNA binding assays using increasing amounts (0.75, 1.5, 3, and 6 µl) of WT (lanes 2–5) or Elp3 C108A mutant (lanes 6–9) Elongator from Fig. 3B and a radioactively labeled, random sequence RNA oligonucleotide (8). A reaction lacking Elongator is shown in lane 1 to indicate migration of free RNA. **C**, RNA immunoprecipitation experiment using α-Myc antibodies to immunoprecipitate Elongator from strains expressing WT or point-mutated (C103A and C108A) Elp3. The relative amount of GAL1 mRNA cross-linked to Elongator in each extract was determined by quantitative PCR after reverse transcription. The signal from an untagged control strain was set to 1, and other values were expressed relative to that. The average of four independent experiments is shown. Error bars indicate S.D.
domain was recently shown to bind iron in the archaeal Elp3 protein (24), but no studies have addressed its role in eukaryotes or, more importantly, in the context of the Elongator complex. Here we investigated the functionality of this motif by a combination of genetic and biochemical approaches. The most important findings we report are the following. First, the FeS cluster is essential for normal Elongator function. Point mutations in the cluster resulted in phenotypes that are indistinguishable from those of the complete \textit{ELP3} gene deletion. Second, the cluster is important for the structural integrity of Elongator as well as for its interactions with accessory factors Kt11 and Kt12. However, the interaction with RNAPII in chromatin was not dramatically affected. Finally we report the development of a novel epitope tag and purification procedure that make it possible to virtually deplete a tagged protein from a crude yeast extract and then release pure protein for biochemical characterization. This procedure is much more reproducible and gives much higher yields than others we have tested.

What is the role of the Elp3 iron-binding module? Our data represent an argument for the idea that the eukaryotic FeS cluster is not a catalytic component of Elp3 (although we cannot completely rule this possibility out) but rather a structural motif required for the integrity of the Elongator complex. Numerous lines of evidence support this hypothesis. First, despite several attempts, no evidence for histone demethylation activity was obtained (see below). Second, purification of Elongator complexes containing point mutations predicted to disrupt the cluster was only possible using the novel highly efficient and rapid purification protocol, so the mutations resulted in significant reduction in the structural integrity of Elongator. Furthermore the mutations disrupted interactions between Elongator and the Kt11 and Kt12 proteins. Finally the mutant complexes retained HAT and RNA binding activity. We thus propose that the FeS cluster is important for the stability of Elongator and its interactions with certain accessory proteins. It is perhaps surprising, therefore, that point-mutated Elongator complexes can be purified at all. However, mutation of conserved cysteines in archaeal Elp3 demonstrated that loss of one residue destabilized the FeS cluster but did not prevent its formation (24), whereas loss of multiple residues completely abrogated iron binding. This synergism would explain our ability to purify at least some intact FeS cluster mutant complexes when using a protocol optimized to prevent oxidation events.

As the starting point for this study, we set out to investigate the possibility that the FeS cluster might be important for Elp3-mediated histone demethylation as suggested by Chinenov (23). However, despite conducting comprehensive analyses using diverse assays, we failed to uncover evidence to support this idea. Elongator was also purified in an oxygen-free tent using the improved protocol to prevent oxidation of the FeS cluster. Large amounts of this protein exhibited no significant histone demethylation activity in various \textit{in vitro} reactions where demethylation was assayed using antibodies against a variety of modified histones or by formaldehyde production (a predicted by-product of catalysis (23)) (supplemental Fig. S2). Furthermore bulk histone methylation (measured by quantitative Western blotting) and levels of histone methylation at specific genomic locations (measured by chromatin immunopre-
cipation) were largely unaffected in elp strains (supplemental Fig. S3).

ELP genes are required for formation of the modified nucleosides mcm²U, mcm³s²U, and mcm³U at the wobble position of certain tRNA species, although a direct role for Elongator in this process has not been demonstrated (13, 14). In addition to the FeS cluster, Elp3 also contains a region homologous to the catalytic domain of proteins belonging to the AdoMet radical family (23). In enzymes of this family, a reduced form of the FeS cluster can donate an electron to AdoMet, generating methionine and a 5′-deoxyadenosyl radical. Such a reaction mechanism could theoretically be involved in the formation of cm²U in tRNA molecules, but currently this idea cannot be tested because we and others have been unable to develop an in vitro assay for this reaction. The AdoMet binding motif, comprised of a glycine-rich region near the FeS cluster (23), is apparently functional in archaenal Elp3 (24). However, despite repeated attempts, we have been unable to detect binding of yeast Elp3 to AdoMet (supplemental Fig. S4). Mutation of the Gly-181 residue within this domain to alanine had no phenotypic defect that we could detect, but mutation to serine resulted in essentially the same phenotypes and lack of structural integrity that were also observed with the FeS cluster mutants (data not shown). Clearly more work needs to be carried out to determine whether Elongator functions directly in tRNA modification and whether the conserved N-terminal motifs are required for this reaction. The available evidence, including that presented here, suggests that, like the FeS cluster, the glycine-rich domain might be required for structural purposes and that its similarity to AdoMet-binding domains could simply be an evolutionary relic. In this respect, it is important to note that no proteins with sequence homology to other Elongator subunits have been found in Archaea, suggesting that the evolution of this complex might have involved a loss of catalytic activity concurrent with a gain in complexity (i.e. subunit composition). However, further studies of the archaenal proteins are warranted to elucidate the function of Elp3-mediated AdoMet cleavage (if any), which may shed more light on this issue. It is also important to stress that although our data propose an alternative function as a structural domain the possibility that the FeS motif also plays a catalytic role cannot be completely ruled out based on negative results alone.

One intriguing possibility is that the FeS cluster is somehow involved in sensing the oxidative state of cells and then signaling this state to basic cellular reactions such as transcription and tRNA modification. In this respect, it is interesting to note that Elongator interacts with the Kti11 protein only in the soluble, DNA-free fraction (which includes the cytoplasm) and that this interaction requires the integrity of the FeS cluster, whereas the interaction with RNAPII in chromatin does not. In fact, the C108A mutant Elongator complex appeared to interact better with RNAPII than did wild type Elongator (Fig. 5C) and even exhibited weak binding to RNAPII in the soluble, chromatin-free fraction (data not shown). Addressing the idea that the FeS motif regulates protein interactions in response to changes in the oxidative state is an important future goal.

The present study further supports the idea that Elongator functions within the context of chromatin. We confirmed its association with RNAPII, and as expected, this interaction was only detectable in chromatin-enriched yeast extracts. This likely explains why earlier studies failed to uncover an interaction between these complexes (34). Furthermore the significant amount of Elongator purified from chromatin compared with soluble fractions highlights its role in DNA-related processes. Future studies of Elongator should focus on possible cross-talk between the cytoplasmic (i.e. tRNA modification) and nuclear (i.e. transcription and histone acetylation) functions of Elongator and how this might be regulated.

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