Molecular Architecture, Structure-Function Relationship, and Importance of the Elp3 Subunit for the RNA Binding of Holo-Elongator*

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The molecular architecture of six-subunit yeast holo-Elongator complex was investigated by the use of immunoprecipitation, two-hybrid interaction mapping, and in vitro studies of binary interactions between individual subunits. Surprisingly, Elp2 is dispensable for the integrity of the holo-Elongator complex, and a purified five-subunit elp2Δ Elongator complex retains histone acetyltransferase activity in vitro. These results indicate that the WD40 repeats in Elp2 are required neither for subunit-subunit interactions within Elongator in vitro for Elongator interaction with histones during catalysis. Elp2 and Elp4 were largely dispensable for the association of Elongator with nascent RNA transcript in vivo. In contrast, Elongator-RNA interaction requires the Elp3 protein. Together, these data shed light on the structure-function relationship of the Elongator complex.

The Elongator complex was first identified as a component of a hyperphosphorylated RNA polymerase II (RNAPII)1 holoenzyme isolated from yeast chromatin (1). One of the subunits of Elongator, Elp3, contains domains conserved among histone acetyltransferases (HATs) (2), and Elongator has HAT activity directed against histone H3 and to a lesser extent H4 in vitro (3), raising the possibility that Elongator acetylates histones during RNAPII transcript elongation.

Elongator HAT activity is essential for Elongator function in vivo as point mutations that abolish the catalytic function of Elp3 in vitro also confer the full range of phenotypes (4). elp mutations include temperature sensitivity at 39 °C, salt sensitivity, and slow adaptation to growth on carbon sources such as galactose (1, 5). In all cases tested, elp phenotypes correlate with a failure of the mutant cells to activate, in a timely manner, the genes required for growth under the new conditions (1).

An elp deletion in combination with the deletion of different genes encoding transcription factors confers synthetic phenotypes. Thus, elp3 in combination with rpb9 (encoding an RNA-PPI subunit) is lethal (8), as is the combination of an elp3 and ctk1 mutation (CTK1 encodes a subunit of the CTD kinase, CTDK1) (9). Cells expressing a conditional allele of SPT16 (encoding the largest subunit of yeast FACT-CP complex) also display a synthetic phenotype in combination with elp3 mutation (10). Recent data have shown that Elongator also genetically interacts with Mediator, Rad6 ubiquitin ligase, Paf1 complex, and Rpd3-Sin3 complex. Finally, combining the mutation of elp3 with gen5 (encoding the catalytic subunit of the SAGA-ADA HAT complexes) confers severe growth defects, which are not seen in either of the single mutants (4). Significantly, the gen5 elp3 double mutant has reduced levels of histone H3 acetylation in several genes compared with the single mutants, and low levels of acetylation in the coding region of these genes correlates with reductions in gene transcription. By contrast, low levels of acetylation in the promoter of genes does not correlate with reduced transcription nor with reduced promoter occupancy by the TATA-binding protein (11). Recent experiments using RNA-immunoprecipitation have shown that Elongator is indeed present in the coding region of active genes in vivo (12).

The Elongator complex was first thought to consist of three subunits, named Elp1, Elp2, and Elp3 (now called core Elongator) (1), but later purification of the complex from a yeast strain expressing epitope-tagged Elp1 has shown that the active complex, holo-Elongator, consists of six subunits (Elp1–Elp6) (13–15), which are organized in two three-subunit subcomplexes (13, 15). Significantly, deletion of any one of the (ELP) genes encoding these six subunits confers more or less identical phenotypes, and new phenotypes are not detected upon concomitant deletion of two or three ELP genes (5, 13). These data suggest a tight functional connection between the proteins comprising the Elongator complex. Consistent with this idea, holo-Elongator, but not core Elongator, has HAT activity even though both complexes contain the catalytic Elp3 subunit (13).

Here we investigate the molecular architecture of Elongator complex and show that the only ELP gene that can be deleted without significant loss of Elongator integrity is ELP2. Surprisingly, an Elongator complex lacking Elp2 even retains the ability to acetylate histones in vitro. We also show that the association of Elongator with nascent RNA in vivo requires Elp3 but not Elp2 or Elp4.

EXPERIMENTAL PROCEDURES

Yeast Strains and Phenotypic Analysis—All Saccharomyces cerevisiae stains used for genetic analysis (Table I) were congenic with strain W303 and were grown and manipulated as described previously (1, 4).

1 The abbreviations used are: RNAPII, RNA polymerase II; HAT, histone acetyltransferase; HA, hemagglutinin; GST, glutathione S-transferase.
Expression of Tagged Proteins in Vivo—For construction of the Elp4-HisHA strain, part of the ELPL4 open-reading frame was amplified using primers 5′-GCCGGGGTGACCCGGCTGGCGCTGGGAACCTCTG-3′ and 5′-CCGGCCGATCTAGTCTAAGATATCTGGTCTC-3′ and cloned into pSE.HISHA-104 (16) using the KpnI and BamHI sites to produce plasmid pELP4-HISHA-104. After yeast transformation, a GST-Elp5 fusion protein, the open reading frame of the ELP5 coding gene was cloned in both −H11003 and −H11006 template used was pBluescript II KS (−) (Stratagene) according to the manufacturer's recommendations. The DNA was purified and immobilized on glutathione beads (Amersham Biosciences) by incubation for 30 min at room temperature. The soluble GST control protein was produced in the same way.

In Vivo Transcribed/Translated Proteins—The recombinant Elp4 and Elp6 proteins used for the in vitro pull-down experiments were produced using the TNT T7-coupled wheat germ extract systems (Promega) according to the manufacturer's recommendations. The DNA used template was pBluescript II KS (+), into which the coding sequence of the two genes was cloned. Details are available on request.

In Vivo Pull-down Experiments—Immobilized baculovirus-expressed histidine-tagged Elp1 or Elp2 and bacterially expressed GST or GST-Elp5 proteins were mixed with the product of in vitro transcription/translation (Elp4 or Elp6) reactions in a buffer containing 250 mM potassium acetate, 100 mM Hepes-KOH, pH 7.6, 20% glycerol, 0.1% Nonidet P-40, 1× proteinase inhibitors, and 4 mM β-mercaptoethanol and incubated overnight. The beads were washed three times before the bound proteins were separated by PAGE on a 10% SDS gel. The Elp4 and Elp6 proteins showed weak nonspecific binding to nickel-agarose. Therefore, these beads were washed three times with the above buffer but containing 30 mM imidazole.

Other Assays—Histone acetyltransferase reactions (15 μl) were carried out as described (3). RNA immunoprecipitation experiments were carried out as described by Gilbert et al. (12).

RESULTS

In a multisubunit protein complex, the stability of an individual subunit can be dependent on the presence or integrity of the other subunits. We examined the amount of individual Elongator subunits present in extracts derived from different elp deletion strains. Fig. 1A (top panels) shows the results of an analysis where whole cell extracts were immunoblotted and probed with anti-Elp3 antibodies. As a loading control, reactivity with an anti-tubulin antibody was used. Interestingly, the Elp3 protein either could not be detected or was present at very low levels in cells lacking the ELP1 gene. In cells lacking any of the other ELP genes, the Elp3 levels were also somewhat reduced, but the protein was still detectable (Fig. 1A, top panel, compare the tubulin/Elp3 ratio in wild type (WT) cells with that in the mutants). By contrast, Elp4, Elp5, and Elp6 were only absent from extracts derived from strains in which their encoding gene was deleted (Fig. 1A, middle and bottom panels). Importantly, Northern blot analysis of RNA from the same cells showed that the mRNA level of the ELP3 gene was comparable in wild type and all of the elpΔ cells (Fig. 1B) suggesting that reduced protein stability rather than gene expression was causing the effect. We conclude that deletion of the ELP1 results in substantial destabilization of the Elp3 protein, suggesting that the Elp1 protein is required for the integrity of Elp3, in all likelihood because of a direct interaction between these proteins.
**Protein-Protein Interactions among Elongator Subunits**—To investigate protein-protein interactions in the Elongator complex, we next performed co-immunoprecipitation experiments in different *elp* deletion strains. For these experiments whole cell extracts were prepared from cells expressing a tagged version of an Elongator subunit while lacking another one, for example from *elp2* cells expressing Elp1-HA. To enable high efficiency immunoprecipitation and to increase specificity, whole cell extracts were first subjected to chromatography on Bio-Rex before being loaded onto 12CA5-conjugated Sepharose A beads. As shown in Fig. 2A, Elongator could, under those conditions, be specifically immunoprecipitated from wild type cell extracts carrying either Elp1 or Elp4 tagged with an HA epitope but not from control cells that did not express a tagged Elp protein. Remarkably, when the *ELP2* gene was deleted, Elp1 still interacted with the five remaining subunits, although the three smaller proteins Elp4, Elp5, and Elp6 appeared to be somewhat substoichiometric (Fig. 2B, *left panel*). This experiment indicates that the Elp2 protein is not required for the integrity of the Elongator complex.

When extracts from cells lacking the *ELP3* gene were used, only the Elp1 protein was detected in the precipitates, which indicates that none of the remaining four subunits interact strongly with Elp1 (Fig. 2B, *middle panel*). We conclude that the Elp3 protein is not only essential for catalytic function but also for the integrity of the Elongator complex.

In the absence of Elp4, the Elp1 protein could still interact with Elp3 (Fig. 2B, *right panel*). However, in this case the Elp2, Elp5, and Elp6 proteins could not be detected even in the inputs. This can be explained in two ways: either those proteins no longer co-elute with Elp1 and Elp3 from Bio-Rex if Elp4 is absent, or deletion of the *ELP4* gene results in reduced Elp2, Elp5, and Elp6 protein levels. Our previous experiments showed that Elp5 and Elp6 protein levels were not decreased significantly in any of the *elp* mutants (Fig. 1A), pointing to a lack of interaction with core Elongator as the likely explanation. Unfortunately, the Elp2 protein could not be detected in this case in the Bio-Rex-70 eluate (and could generally not be detected in crude whole cell extracts with our polyclonal anti-Elp2 antibody), precluding any conclusions on the fate of this protein in *elp4* extracts. We conclude that Elp4 is required for the association of Elp2 and the small Elp4/5/6 complex with Elp1 and Elp3.

When extracts from *elp5* cells expressing Elp4-HA protein were used, Elp4 only co-immunoprecipitated Elp6 and low amounts of Elp3 (Fig. 2C, *left panel*). This suggests that Elp4 interacts directly with Elp6 and that neither of these two proteins interacts strongly with any of the three larger proteins Elp1, Elp2, and Elp3, although a weak Elp4/Elp6-Elp3 interaction was evident. The absence of a signal for Elp1, even in the input, again precluded firm conclusions on the fate of this protein in *elp5* cells. However, it was obvious from the previous experiment with *elp3Δ* cells that Elp1 does not interact directly with Elp4 or Elp6 (Fig. 2B, *middle panel*). In *elp6* cells expressing Elp4-HA protein, Elp4 also only co-precipitated a small amount of Elp3 (Fig. 2C, *right panel*), supporting the notion that these proteins interact directly, albeit weakly. Elp4 did not interact with Elp5 in the absence of Elp6. As Elp5 is stable in *elp6* cells (Fig. 1A, *middle panels*), this indicates that it is incorporated into the small subcomplex in an Elp6-dependent manner.

We conclude from the above experiments that there is a direct interaction between Elp1 and Elp3, as well as between Elp4 and Elp6. Because a core-Elongator complex consisting of
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Fig. 3. Binary Elongator subunit interactions revealed by two-hybrid interaction or direct protein-protein interaction mapping in vitro. A, Elp4 and Elp6 interaction shown by two-hybrid interaction. Positive and negative controls were from the Clontech matchmaker kit. Please note that for the large number of negative interaction results we obtained, it was ensured that the relevant Elp-Gal fusion protein rescued the mutant phenotype of the corresponding elp strain, showing that the Elp protein in question was correctly folded and functional even in the presence of the Gal moiety. B, protein-protein interactions investigated with recombinant Elongator subunits. Upper left panel, Elp1 does not directly interact with Elp4 and Elp6. Upper right panel, Elp2 does not directly interact with Elp4 and Elp6. Lower panels, Elp5 interacts directly with Elp6.

Elp1, Elp2, and Elp3 has previously been isolated (1), the absence Elp2 in Elp1-precipitates from elp3 cells also indicate that interactions with Elp3 are required for the incorporation of Elp2 into core-Elongator. Surprisingly, the co-immunoprecipitation experiments do not indicate any strong direct binary interactions between any individual protein in the Elp4/5/6 module and any one of the proteins in core Elongator. This suggests that it is primarily novel interaction surfaces created by the association of Elp1, Elp2, and Elp3 in core-Elongator, and Elp4, Elp5, and Elp6 in the small subcomplex, respectively, that are important for the later association of these subassemblies into holo-Elongator.

Pairwise Elongator Protein Interactions—To compliment the co-immunoprecipitation studies, pairwise interactions between individual Elongator proteins were examined by use of the yeast two-hybrid system. The genes encoding all the Elongator subunits were cloned in-frame with the activation domain and the DNA-binding domains of the GAL4 activator, respectively, and tester yeast strains were transformed with combinations of the DNA-binding domains of the GAL4 activator, respectively, and transcribed/translated in vitro. Unfortunately, despite attempts in several expression systems, we were unable to obtain soluble recombinant or in vitro transcribed/translated Elp3 protein. Examples of the results obtained are shown in Fig. 3B. Of all of the many combinations that were tested, only a direct interaction between Elp5 and Elp6 was detected.

Taken together, these results make it possible to propose a model to describe the molecular architecture of the Elongator complex (Fig. 4). In the large core-Elongator subcomplex, Elp1 protein interacts with Elp3 but does not interact strongly with Elp2 in the absence of Elp3. This suggests a direct Elp2-Elp3 interaction. On the other hand, the small subcomplex is formed based on direct interactions between Elp4 and Elp6 and between Elp6 and Elp5. Although not absolutely required, the Elp2 protein serves to somewhat stabilize the core Elongator-Elp4/5/6 interaction, whereas Elp3 is essential for the integrity of the complex. Furthermore, a weak interaction between Elp3 and Elp4 was detected. Based on these results we suggest that, rather than relying solely on strong binary interactions between individual subunits of the respective subcomplexes, the formation of the small subcomplex creates new interaction surfaces to enable contacts with Elp3 and Elp2, as well as with novel interaction surfaces created by the association of Elp1, Elp2, and Elp3 into core-Elongator (Fig. 4).

The WD40 Repeat Protein, Elp2, Is Dispensable for the in Vitro HAT Activity of Elongator—The data presented above revealed that although an elp2 strain displayed the typical Elongator phenotypes (5), the remaining five subunits still exist as a complex in these cells. To investigate whether the five-subunit elp2Δ complex also retained Elongator activity, we purified the “mutant” complex and tested its in vitro HAT activity. This was particularly relevant, because several HAT and histone deacetylase complexes harbor subunits, which, like Elp2, contain WD40 repeats. It has thus been suggested that WD40 repeats might be involved in contacting histones to facilitate the acetylation transfer and deacetylation reactions, respectively (18, 19). The purification scheme employed was identical to that used previously for the purification of a six-subunit Elongator complex from cells carrying a double affinity-tagged Elp1 protein (13). As indicated by Fig. 5A, a purified elp2Δ Elongator complex from two independent purifications retained the five other subunits, although the three smaller subunits as well as the Elp3 protein appeared to be somewhat substoichiometric compared with the wild type complex. To be able to compare the HAT activity of the elp2Δ complex with that purified from wild type cells, we therefore used similar amounts of the catalytic Elp3 subunit in the HAT reactions, with the consequence that other subunits, particularly Elp1, were present in larger amounts in the reactions containing the mutant five-subunit complex. Fig. 5B shows the result of HAT assays comparing wild type and the two independently purified mutant complexes. Surprisingly, Elongator retained in vitro HAT activity directed against H3 even in the absence of Elp2. Adding recombinant, His-tagged Elp2 to the reactions did not
dramatically affect the activity (the apparent stimulation of wild type complex (Fig. 5B, lanes 1 and 2) was because of loading differences). These data demonstrate that the WD40 repeat protein Elp2 is not required for Elongator HAT activity in vitro.

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**Fig. 5.** Five-subunit elp2 Elongator complex retains HAT activity in vitro. A, two independently purified elp2 Elongator complexes and their subunit stoichiometry compared with wild type (WT). The Elp2 subunit is present only in wild type, but this Western blot is not shown. B, HAT activity of wild type and mutant Elongator complex with or without the addition of Elp2 as indicated. Please note that the activity of Elongator in HAT assays differs significantly from preparation to preparation and with freeze-thawing. It is therefore not possible to make a definitive judgment on the possible minor quantitative differences in activity, if any, between wild type and mutant Elongator.

**DISCUSSION**

The results presented here represent the first comprehensive investigation of the structure-function relationship of Elongator, a histone acetyltransferase complex with a role in RNAPII transcription. Our results reveal the detailed molecular architecture of the complex and suggest roles, or unexpected lack of roles, for individual subunits.

Previous work (13–15) showed that holo-Elongator is composed of two weakly associated subcomplexes. Subsequent
work by Schaffrath and co-workers (20, 21) using immunoprecipitation of tagged Elongator subunits and detection of a coprecipitated differently tagged subunit gave some information about subunit requirements for Elongator stability. Although the present work confirms and significantly extends the conclusions derived from these studies, there are also surprising contradictions. For example, Frohloff et al. (21) detected the Elp3 protein in cells lacking ELPI, whereas our results indicate that normal Elp3 stability in vivo requires ELPI (Fig. 1). Likewise, these authors concluded that the structural integrity of the small Elp4/5/6 subcomplex requires the ELPA, ELPB, and ELPC genes, whereas our work shows that Elp4 can interact with Elp6 in the absence of ELPA (Fig. 2C). The reasons for these differences are presently unclear.

One of the theses for the architecture of the Elongator complex before starting our studies was that the WD40 repeat-containing Elp2 protein would turn out to either be essential for the integrity of the Elongator complex, or be required for its HAT activity. WD40 repeats are protein-protein interaction domains, and WD40-repeat proteins have been identified in other histone modifying/interacting proteins (18, 19). Surprisingly, Elp2 turned out to be dispensable for both; a five-subunit other histone modifying/interacting proteins (18, 19). Surpris-
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for the integrity of the Elongator complex, or be required for its
between RNAPII and Elongator. For example, the protein was
protein, Kti12/Tot4 has been proposed to bridge interactions be-
proteins, such as Kti12/Tot4. The Elongator-interacting pro-
that the role of Elp2 might be to allow interactions with other
(Fig. 6). Data from Schaffrath and co-workers (20) suggests
these differences are presently unclear.

In contrast to the Elp2 protein, the Elp3 subunit appears to
play a crucial role for all Elongator functions. It is the catalytic
subunit (2), it is crucial for the integrity of the holo-Elongator
complex (Fig. 2), and it is essential for RNA binding (Fig. 6). In
light of the fact that Elp3, but not the other subunits, is con-
erved from Archaea to man (2), this is perhaps not surprising.
It thus seems reasonable to expect that the fundamental func-
tions of the Elongator complex be supplied by the abilities intrinsic to Elp3 with the other subunits playing primarily
function/augmentative or regulatory roles.

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