Physical and Functional Interaction between Elongator and the Chromatin-associated Kti12 Protein*

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Cells lacking KTI12 or Elongator (ELP) genes are insensitive to the toxin zymocin and also share more general phenotypes. Moreover, data from low stringency immunoprecipitation experiments suggest that Elongator and Kti12 may interact. However, the precise relationship between these factors has not been determined. Here we use a variety of approaches to investigate the possibility that Elongator and Kti12 functionally overlap. Native Kti12 purified to virtual homogeneity under stringent conditions is a single polypeptide, but depletion of Kti12 from a yeast extract results in co-depletion of Elongator, indicating that these factors do interact. Indeed, biochemical evidence suggests that Elongator and Kti12 form a fragile complex under physiological salt conditions. Purified Kti12 does not affect Elongator histone acetyltransferase activity in vitro. However, a variety of genetic experiments comparing the effects of mutation in ELP3 and KTI12 alone and in combination with other transcription factor mutations clearly demonstrate a significant functional overlap between Elongator and Kti12 in vivo. Intriguingly, chromatin immunoprecipitation experiments show that Kti12 is associated with chromatin throughout the genome, even in non-transcribed regions and in the absence of Elongator. Conversely, RNA-immunoprecipitation experiments indicate that Kti12 only plays a minor role for Elongator association with active genes. Together, these experiments indicate a close physical and functional relationship between Elongator and the highly conserved Kti12 protein.

In competing for limited resources, microorganisms have evolved sophisticated strategies to gain selective advantage over their competitors. One of these is the secretion of toxic compounds that results in the killing or growth arrest of other species or genera. The yeast Kluyveromyces lactis secretes a toxin, referred to as zymocin, which inhibits the growth of various sensitive yeast genera, including Saccharomyces cerevisiae (1). The native toxin is a heterotrimeric (αβγ) structure composed of three subunits, two of which are involved in facilitating toxin entry. Cytotoxicity resides solely within the γ subunit, and intracellular expression of this subunit alone in S. cerevisiae abrogates growth (2). Genetic screening for mutations that confer resistance toward the intracellular expression of the zymocin γ subunit identified genes that were named TOT1–7 (toxin target) (3–5). Interestingly, the initially isolated genes were found to encode subunits of either the yeast Elongator (Elp1/Tot1/Iki3, Elp2/Tot2, Elp3/Tot3, Elp4/Tot7, Elp5/Tot5/Iki1, and Elp6/Tot6) or the Kti12 protein (Tot4). ELP1/IKI3 and KTI12 were also isolated previously in independent screens for mutants that render cells resistant to the native toxin (Insensitive to Killer (IKI) and killer toxin-insensitive (KTI) genes, respectively) (2, 6).

Elongator was first biochemically characterized as a component of the elongating form of RNA polymerase II (RNAPII) (7) and contains the highly conserved histone acetyltransferase (HAT) Elp3 (8). Recent evidence from both yeast and human cells indicates that Elongator interacts with active genes in vivo (9, 10). Interestingly, point mutations in Elp3, which abolish its HAT activity, also confer toxin resistance (5, 11). Moreover, mutagenesis studies on the ELP3 gene identified mutations outside the HAT domain, which confer sensitivity to killer toxin, but not the phenotypes otherwise typical of elp strains (separation of function mutations) (3). This suggests that the requirement of Elongator for γ toxin sensitivity can be genetically dissociated from general Elongator function, perhaps through abolishing direct toxin–Elp3 interactions. The deletion of other HAT-encoding genes such as SAS3, HPA3, HAT1, and GCN5 does not confer zymocin resistance, further suggesting that Elp3, and not just any cellular HAT activity, is a target of the toxin (12).

In contrast to the deletion of ELP genes, which confers zymocin resistance, deletion of several genes encoding subunits of RNAPII transcription-related complexes renders cells toxin-hypersensitive (12). This is true for genes that encode subunits of the SAGA, the SWI/SNF, the Mediator, and the Ccr4-Not complexes. Zymocin hypersensitivity is also observed in cells carrying deletions of transcript elongation-related factors such as ctk1 (RNAPII C-terminal domain (CTD) kinase 1), fcp1 (CTD phosphatase), and rrf1 (Paf complex) or mutations in rpb2 (RNAPII). In contrast, histone deacetylase-defective cells display either wild type or even reduced zymocin sensitivity. Based on the latter finding, Kitamoto et al. suggested that situations favoring histone hyperacetylation might reduce the cellular requirement for the HAT activity of Elongator and thereby reduce zymocin toxicity (12).

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The abbreviations used are: RNAPII, RNA polymerase II; CTD, C-terminal domain; ChIP, chromatin immunoprecipitation; GST, glutathione S-transferase; HA, hemagglutinin; HAT, histone acetyltransferase; RIP, RNA immunoprecipitation.
The above data suggest that the effect of the toxin is on RNAPII-dependent transcription. In apparent agreement with this idea, low resolution hybridization showed that global poly(A)^+ mRNA levels generally decline in the presence of zymocin, and Northern blot analysis showed significantly reduced levels of specific RNAPII-generated transcripts (3, 5). In an attempt to further reinforce the notion that zymocin action is linked to RNAPII, Jablonowski and Schaffrath studied the effects on zymocin toxicity of genetic conditions that would be supposed to directly impair polymerase activity, such as mutations in the RNAPII kinase encoded by the BUR1/BUR2 genes, deletion of the SRB10 CTD kinase gene, and inactivation of the kinase activity of TFIH (13). In all cases, the mutant cells exhibited zymocin hypersensitivity. Moreover, hypersensitivity was also caused by truncation of the RNAPII CTD itself, suggesting that Kti12 occupies the promoter, but not the coding region, of the gene (15). As mentioned above, KTI12 and the ELP genes were identified in a genetic screen using intracellular expression of the y gene subunit as a means to look for targets of the toxin gene (TOT) (5). Cells lacking the KTI12/TOT4 gene display temperature sensitivity and 6-azauracil sensitivity as well as hypersensitivity to Calcofluor White and caffeine (4). Thus, kti12 cells have phenotypes that are similar to those observed for elp cells (4, 7). Low stringency co-immunoprecipitation experiments suggested the existence of an interaction between Kti12 and Elongator as well as one between Kti12 and the form of RNAP II phosphorylated at serine 5 of the CTD repeat (4, 14). However, deletion of KTI12 does not appear to affect the structural integrity of six-subunit Elongator complex (4), making it unlikely that Kti12 is a structural component of Elongator. Interestingly, chromatin immunoprecipitation (ChIP) experiments suggested that Kti12 occupies the promoter, but not the coding region, of the ADH1 gene (15).

We were intrigued by the data suggesting a functional connection between Kti12 and Elongator. Our previous data showed no evidence for Kti12 in highly purified Elongator fractions (11), and the apparent overlap of elp and kti12 phenotypes might conceivably be misleading. Moreover, the immunoprecipitation experiments suggesting a Kti12-Elongator interaction were performed under non-stringent conditions that permit detection also of less meaningful protein-protein interactions. In this paper we set out to investigate in more detail the possibility that Elongator and Kti12 functionally overlap. Our data are consistent with the idea that Elongator and Kti12 interact in a manner that has important consequences for the function of Elongator as a histone acetyltransferase in vivo. Our data also suggest that Kti12 is a general chromatin component rather than a promoter-specific or even a gene-specific factor as proposed previously (15).

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Phenotypic Analysis**—All *S. cerevisiae* stains used for genetic analysis were congenic with strain W303 and grown and manipulated as described previously (7, 16). Genotypes of the strains used are shown in Table I.

**Expression of Tagged Proteins in Vivo**—For the construction of the Kti12-His6HA strain, part of the KTI12 open reading frame was amplified by PCR and cloned into pSE.HISHA-304 (17) using the KpnI and BamHI sites to produce plasmid pKTI12-HISHA-304. After yeast transformation, a TRP^+^ clone was isolated in which the 3′-end of the KTI12 gene was replaced, resulting in expression of a Kti12-(His)_6^-HA fusion protein. Phenotypic analysis showed that the (His)_6^-HA epitope tag did not interfere with Kti12 function (data not shown). Similar procedures, but using tagging plasmids kindly supplied by Dr. Kim Nasmyth (18), were used to produce and characterize Kti12–6HA and Kti12–18Myc strains (oligonucleotide sequences and other details are available on request).

**Protein Purification**—The procedure for purification of Kti12 from a *K. lactis* strain has been described elsewhere (11, 17). Gel filtration analysis was performed using a Superose 6 column (Amersham Biosciences) connected to a Biologic fast protein liquid chromatography system (Bio-Rad). The buffer used was 2% glycerol, 250 mM potassium acetate, pH 7.6, 0.1% Nonidet P-40, and 1× protease inhibitors. The protein-containing fraction was then dialyzed against the same buffer and run immediately before and after each experimental sample for reference.

**Protein Identification**—Gel-fractionated proteins were digested with trypsin and peptides analyzed by matrix-assisted laser-desorption/ionization reflectron time-of-flight mass spectrometry (MALDI-TOF) and by electrospray ionization tandem mass spectrometry as previously described (17). Selected mass values from the MALDI-TOF experiments were taken to search the protein non-redundant data base (National Center for Biotechnology Information, Bethesda, MD) using the PeptideSearch (19) algorithm. Tandem mass spectrometry spectra were inspected for y^+^ ion series to compare with the computer-generated

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**Table I**

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**K. lactis** strains

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*Kind gift from Prof. Michael J. R. Stark.*
Elongator and Chromatin-associated Kti12 Protein Interaction

fragment ion series of the predicted tryptic peptides.

Co-immunoprecipitation Experiments—500 μg of yeast whole cell extract in buffer A (40 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 20% (v/v) glycerol, and protein inhibitor mix) containing 250 or 500 mM potassium acetate, as indicated, was incubated for 2 h with Sepharose A beads, which had been previously conjugated with the 12CA5 antibody. After incubation, the beads were washed three times with the same buffer, re-suspended in 1× SDS loading buffer, and the bound proteins were subjected to SDS-PAGE and Western blot analysis.

Expression of GST-Kti12 in Bacteria and Antibody Production—The Kti12 open reading frame was cloned in-frame with the GST protein in pGEX-3X and the fusion protein expressed in Escherichia coli BL21 DE3 cells by induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 6 h at 28 °C. Subsequently, cells were lysed in phosphate-buffered saline, and inclusion bodies were solubilized by sonication of the pellet in the presence of 0.5% sarcosyl. Solubilized GST-Kti12 was purified on glutathione-Sepharose per the manufacturer’s instructions (Amersham Biosciences). The recombinant fusion protein was used to immunize rabbits (Imgenex). The resulting antibody was used for Western blots at 1:1000 final dilution in phosphate-buffered saline containing 0.05% Tween and 5% (w/v) milk powder.

Killer Toxin Assays—To analyze killer toxin sensitivity, K. lactis cells expressing or not expressing the zymocin toxin were left to grow overnight on YPD (1% yeast extract, 2% peptone, and 2% dextrose) medium (strains used are shown in Table I). The next day, ~10,000 S. cerevisiae cells were dissolved in water and spotted in the vicinity of the growing K. lactis cells. The growth of the different mutants was compared with the characteristic eclipse growth of wild type S. cerevisiae cells.

Other Assays—Histone acetyltransferase reactions (30 μl) were carried out as described (11). Chromatin and RNA immunoprecipitation experiments were performed as described (9, 20, 21). Oligonucleotide primer sequences are available on request.

RESULTS

Purification of Native Yeast Kti12—Previous work by Schaffrath and co-workers reported evidence suggesting an Elongator-Kti12 interaction (4). However, whether a small subset of Elongator complexes contained Kti12 as an integral, tightly associated subunit or whether Kti12 was merely weakly interacting with Elongator remained unclear. Moreover, the experiments suggesting the interaction were performed under very low stringency conditions (60 mM sodium acetate) (4), which might result in the detection of interactions that are not biologically significant.

We tested the possibility that Kti12 and Elongator might exist in the same complex by isolating native yeast Kti12. The genomic KTI12 gene was modified so that the expressed Kti12 protein carried a C-terminal (His)_10-HA tag. After genetic confirmation that the epitope tag did not interfere with Kti12 function (data not shown), the protein was purified by a mixture of conventional and affinity chromatography (Fig. 1A), as described previously (11, 17). Fig. 1B shows a Coomassie-stained SDS-polyacrylamide gel of highly purified Kti12. The two polypeptides, of 40 and 180 kDa, respectively, were identified by mass spectrometry. As expected, the 40-kDa protein was Kti12. The 180-kDa polypeptide was identified as the product of the YDL223C gene. This protein is a frequent, irrelevant contaminant when this purification procedure is utilized (22).

A rabbit anti-Kti12 antibody, but not the corresponding rabbit pre-bled, recognized the highly purified Kti12-HisHA protein isolated from yeast as well as the recombinant, bacterially expressed GST-Kti12 protein (Fig. 1C, anti-Kti12, lanes 2 and 3, respectively). Using this antibody, we also found that the highly purified holo-Elongator complex did not contain Kti12 (Fig. 1C, anti-Kti12, lane 1). Conversely, the highly purified yeast Kti12 did not contain Elongator, as indicated by the absence of Elp3 and Elp4 (Fig. 1D, lane 2).

These data indicate that Kti12 is not a tightly associated, integral subunit of holo-Elongator complexes in general. Moreover, the finding that purification of Kti12 to virtual homogeneity did not uncover co-purifying proteins indicates that this protein is also not a stable component of other protein complexes.

Elongator and Kti12 Interact in a Salt-labile Manner—The method used to purify holo-Elongator and Kti12 made it impossible to exclude the possibility that a weak, salt-labile interaction exists between these proteins. To investigate possible Kti12-Elongator interactions, co-immunoprecipitation experiments were performed using extracts from cells expressing a version of the Kti12 protein that carried a 6× HA affinity tag. Fig. 2 shows the Western blot analysis of immunoprecipitations with 12CA5 (anti-HA) antibody using extracts from cells expressing untagged and tagged Kti12, respectively. In the control immunoprecipitation from untagged cells, none of the Elongator proteins were detected in the precipitates (Fig. 2A, lane 3), whereas 6× HA-tagged Kti12 effectively co-immunoprecipitated Elp3 and Elp4 under low salt conditions (Fig. 2, lane 6, 250 mM potassium acetate). Under more stringent conditions (Fig. 2, lane 8, 500 mM potassium acetate), there was significantly less Elongator associated with Kti12. However, both Elp3 and Kti12 were clearly co-depleted from the resin flow-through under both conditions (Fig. 2A, compare lanes 5 and 7 with lane 4), suggesting that Elongator and Kti12 do indeed interact but dissociate during the salt wash.

Based on these observations, we now sought to purify the Kti12-HisHA protein under less stringent conditions (250 mM salt) (Fig. 3). In particular, as the majority of Kti12 elutes from Bio-Rex in 600 mM salt, this fraction was diluted to 250 mM prior to loading on 12CA5-conjugated Sepharose A beads (Fig. 1A). After washing this column with buffer containing 250 mM potassium acetate, proteins were eluted and subjected immediately to nickel-agarose affinity chromatography. The resulting nickel-agarose elution profile was examined by Western

FIG. 1. Purification of Kti12. A, schematic overview of purification procedure. B, highly purified Kti12-HA-His. Asterisk denotes common contaminant when using this purification protocol. M, marker lane. C, cross-reactivity of 1.7 pmol of highly purified Elongator (lanes 1), 3 pmol of Kti12-HA-His (lanes 2), and 1.5 pmol of purified recombinant GST-Kti12 (lanes 3) with anti-Kti12 antibodies (Anti-Kti12) or the corresponding rabbit pre-bled (pre-bleed) was investigated by Western blotting. D, as for panel C, but using anti-Elp4 (11) and anti-Elp3 (8) antibodies, respectively.
Western blotting using antibodies directed against the proteins specified on the right. IN, input; FT, resin flow-through; IP, immunoprecipitated material.

Elongator and Kti12 form a salt-labile complex. A, Kti12-HAHis was purified as indicated in Fig. 1A, except that the salt concentration was kept below 250 mM potassium acetate in all steps after Bio-Rex 70. Fractions eluting from nickel-agarose were subjected to Western blotting using antibodies directed against the proteins specified on the right. IN, input; FT, nickel-agarose flow-through; W, wash; 10, 50, and 500 indicate 10, 50, and 500 mM imidazole eluates. The identity of the slower migrating band in the 50 mM imidazole eluate of the Elp3 blot is unknown but may be a modified form of Elp3. B, proteins from the 500 mM imidazole eluate were subjected to filtration through Superox 6. The input and the indicated fractions were analyzed by Western blotting using antibodies against the proteins indicated on the right. Elution of size markers is indicated below the blots.

Genetic Interactions between Kti12 and Gcn5—The above results indicate that Elongator and Kti12 interact physically. Previous genetic characterization has shown that strains lacking KTI12 share number of general phenotypes with strains lacking ELP3 genes (4). We set out to more precisely define the functional overlap between Elongator and Kti12 in vivo. We hypothesized that if Kti12 plays a role in the same cellular pathway as Elongator, then deletion of KTI12 should not result in any further deterioration in the growth of an elp3 strain. Indeed, cells lacking both ELP3 and KTI12 (elp3 kti12) displayed growth rates that were no worse than the kti12 and elp3 single mutants under a number of different conditions (Fig. 4A, and data not shown). We also surmised that if Kti12 is an important functional partner of Elongator in the cell, it should genetically interact with Gcn5, as Elongator does (16, 21). The gcn5 elp3 double mutant displays a number of severe growth defects, such as pronounced temperature sensitivity at 37 °C (16). A gcn5 kti12 double mutant was constructed, and its growth at 37 °C was compared with that of the gcn5 elp3 mutant. Fig. 4A shows that gcn5 elp3 and gcn5 kti12 cells are temperature-sensitive to a similar extent. Significantly, the triple mutant lacking KTI12, GCN5, and ELP3 showed the same growth deficiencies as the gcn5 elp3 double mutant (Fig. 4B), strongly supporting the idea that KTI12 and ELP3 participate in the same genetic pathway.

Finally, the specificity and relevance of the functional overlap between KTI12 and ELP3 was confirmed by suppression analysis. We reported previously that the deletion of a unique combination of histone deacetylases (HOS2 and HDA1) is capable of suppressing the severe gcn5 elp3 phenotype (16). Strikingly, comitant deletion of HOS2 and HDA1 also suppressed the gcn5 kti12 phenotype (Fig. 4C), providing further evidence for a close functional connection between Kti12 and Elp3.

KTI12 and all the ELP genes were isolated in a genetic screen for mutations rendering S. cerevisiae cells insensitive to the zymocin toxin (5). It was also reported that deletion of a number of different transcription-related factors confers zymocin hypersensitivity, thereby genetically linking toxin function with transcription. In particular, gcn5 cells were found to be
even more zymocin-sensitive than wild type cells (12). Therefore, it was of interest to test the effect of GCN5 deletion on the killer toxin insensitivity of kti12 and elp3 (Fig. 5). As expected, gcna5 single mutant cells showed great sensitivity toward the toxin (12). Interestingly, however, deletion of either ELP3 or KTI12 in the gcna5 background completely suppressed this sensitivity, supporting the view that Elongator and Kti12 are both primary mediators of zymocin action (Fig. 5). Taken together, these genetic results indicate a significant functional overlap between Elongator and Kti12 and suggest that Kti12 function is required for the normal function of Elongator in vivo.

**KTI12 Deletion Affects Histone Acetylation Levels in Vivo**—We showed previously that the severe growth phenotypes of the elp3 gcna5 double mutant correlate with hypoacetylation of histone H3 in a number of genes. Thus, whereas only a minor acetylation defect was observed in the elp3 single mutant, acetylation levels were dramatically reduced at several genes in the gcna5 elp3 double mutant (21). Inspired by this finding, the possibility that the gcna5 KTI12 double mutant cells also display severe hypoacetylation of histone H3 was investigated by ChIP. The level of acetylation at lysine 27 of histone H3 in wild type, kti12, gcna5 gcna5 kti12, and elp3 gcna5 cells was compared (Fig. 6). Significantly, deletion of KTI12 alone resulted in a 50% reduction of acetylation of lysine 27 in the coding region of the BAT1 and SSA4 genes (Fig. 6A). In cells lacking only the GCN5 gene, the acetylation level of lysine 27 was reduced even more dramatically (Fig. 6B). Most significant, the gcna5 kti12 and elp3 gcna5 double mutants both displayed another 2- to 3-fold reduction of the acetylation level of histone H3 lysine 27 compared with the gcna5 single mutant cells (Fig. 6B). In an extension of the genetic data presented above, these results demonstrate a striking similarity between the effects of ELP3 and KTI12 deletion at the molecular level in vivo and are in agreement with the idea that Kti12 affects the HAT activity of Elongator in cells.

**Kti12 Is Dispensable for the in Vitro HAT Activity of Elongator**—Based on the described physical, genetic, and functional interaction between Elongator and Kti12, it was relevant to study whether purified Kti12 had an effect on the in vitro HAT activity of Elongator. Therefore, in vitro HAT assays were performed using highly purified Elongator, with or without the addition of Kti12 (Fig. 7). As expected, holo-Elongator acetylated histone H3 (Fig. 7, lane 1), whereas Kti12 had no HAT activity on its own (Fig. 7, lanes 4–5, and data not shown). No obvious dramatic effect (stimulatory or inhibitory) was detected when increasing amounts of Kti12 were added in the presence of Elongator (Fig. 7, lanes 2–3, and data not shown). These data show that Kti12 does not directly regulate the HAT activity of Elongator in vitro, at least under the conditions tested. Moreover, in agreement with the fact that Kti12 does not contain any motifs that suggest it might be a histone acetyltransferase, the protein itself has no such activity in vitro.

**Kti12 Has Little Effect on the Ability of Elongator to Associate with an Active Gene in Vivo**—We have shown previously that Elongator associates with the nascent mRNA protruding from transcribing RNAPII in vivo (9, 20). To test whether Kti12 is required for the association of Elongator with RNA, RNA immunoprecipitation (RIP) experiments were performed with wild type or kti12 mutant cells (Fig. 8). 9E10 (anti-Myc) immunoprecipitation efficiently co-immunoprecipitated GAL1 mRNA from cells expressing Myc-tagged, but not untagged, Elp1. In the ab-
The integrity of Elongator (20). The promoter of that Elp2 interacts with Kti12 (15); second, Elp3 is crucial for relevance for two reasons. First, it was suggested previously specifically associated with the promoter of an active gene as suggested by the results of Fichtner (15) that Kti12 is a general chromatin component rather than species, and data not shown). This surprising observation suggests A 9 similarly enriched in these precipitates. In general, Kti12 as- and even the non-transcribed region of chromosome 4 were also precipitated with wild type, and even the non-transcribed region of chromosome 4 was used for the amplification of DNA from Myc-immunoprecipitates. B, as for panel A, but using extracts from wild type and elp2 and elp3 cells. This figure is representative of two independent experiments.

Importantly, deletion of ELP2 or ELP3 only had a minor effect on the recruitment of Kti12 to the tested genomic regions (Fig. 9B). Together, these results suggest that Kti12 is recruited to chromatin in a manner that neither requires Elongator nor gene-activity.

**DISCUSSION**

Multi-subunit complexes often consist of strongly associated core subunits and weakly associated additional subunits. The existence of such weakly associated subunits sometimes makes it hard to distinguish whether a particular protein is a bona fide component of a complex or merely an interacting protein. The data presented here provide compelling evidence for the idea that Kti12 is an important regulator of Elongator function that physically interacts with the complex without being a subunit. Inasmuch as Kti12 and Elongator are highly conserved through evolution, these data suggest that their functional interrelationship is conserved and important across species.

**Biochemical Relationship between Kti12 and Elongator**

The data indicating that Kti12 is not a bona fide Elongator subunit are the following. First, Kti12 purified to near homogeneity is a single polypeptide. Conversely, highly purified Elongator does not contain even small amounts of Kti12. Second, the absence of Kti12 does not affect the integrity or subunit composition of Elongator (4, 11). Finally, in contrast to overexpression of Elongator genes, overexpression of Kti12 leads to killer toxin insensitivity (4).

Nevertheless, the data presented here provide strong support for the idea that Kti12 physically interacts with Elongator.

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\(^2\) T. G. Petrakis and J. Q. Svejstrup, unpublished data.
Most importantly, immunodepletion of Kti12 from crude yeast extracts co-depletes Elongator, but the association is broken in high salt or merely by dilution. Data suggesting that Kti12 might physically interact with Elongator has also been presented by Schaffrath and co-workers (4).

An Important Functional Connection between Elongator and Kti12—The most compelling evidence for an important functional interaction between Kti12 and Elongator comes from genetic experiments. Our data thus demonstrate that a KTI12 deletion virtually phenocopies Elongator (ELP) mutation in several specific and important respects. First, deletion of KTI12 in an elp3 background does not result in an aggravation of the elp3 phenotype. Actually, elp3 kti12 double mutants grow somewhat better than elp3 single mutants, which might suggest that the chromatin-associated Kti12 protein is to some extent detrimental in the absence of Elongator. In any case, these data suggest that Kti12 and Elp3 affect cellular processes through the same molecular pathway and in a very similar way. This conclusion is supported by the finding that kti12 gcn5 and elp3 gcn5 mutants have similar growth phenotypes, and in both cases these phenotypes can be suppressed by deletion of the histone deacetylases HOS2 and HDA1. Both ELP3 and KTI12 deletions also suppress the zymocin hypersensitivity of gcn5 cells. Complementary evidence for genetic similarities between strains lacking KTI12 and ELP genes has also been obtained by others (5). Second, deletion of KTI12 leads to a decrease in histone acetylation in chromatin and also decreases further the more dramatic effect observed upon the deletion of GCN5. More or less identical effects on acetylation were observed previously upon the deletion of ELP3 (21), strongly supporting the idea that Kti12 does not itself have HAT activity, nor does it stimulate Elongator HAT activity in vitro. The specific molecular role played by Kti12 in regulating Elongator HAT function thus remains unclear.

Kti12 Is Associated with Chromatin—in light of the fact that Kti12 and Elongator interact and have more or less identical phenotypes, the finding that Kti12 is associated with chromatin is both interesting and important. It strongly supports the idea that Elongator and Kti12 perform their function in the nucleus. Like Elongator, Kti12 is found in both the cytoplasm and the nucleus (15). The association of Kti12 with DNA as demonstrated by the results of chromatin immunoprecipitation experiments thus indirectly support the RIP data showing that Elongator performs its function in the nucleus (9). Interestingly, our Elongator RIP experiments indicate that Kti12 is not required for the association of Elongator with active genes. Conversely, Kti12 remains chromatin-associated even in the absence of Elongator, underlining the conclusion that although Kti12 and Elongator are functionally closely connected, they are biophysically separate factors. Interestingly, preliminary results from interaction studies using purified, recombinant GST-Kti12 and histone octamers suggest that Kti12 does not bind unmodified histones in vitro.3 Intriguingly, Kti12 interacts with chromatin throughout the genome and not only near the promoter of genes as suggested previously by the evidence of others (15). The biochemical mechanism underlying Kti12 association with chromatin and exactly how this association affects Elongator function remain unknown, but its elucidation is an important subject for future experimentation.

REFERENCES

Physical and Functional Interaction between Elongator and the Chromatin-associated Kti12 Protein
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