RNA Polymerase II Elongator Holoenzyme Is Composed of Two Discrete Subcomplexes*

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Elongator is a histone acetyltransferase complex that associates with the elongating form of RNA polymerase II. We purified Elongator to virtual homogeneity via a rapid three-step procedure based largely on affinity chromatography. The purified factor, holo-Elongator, is a labile six-subunit factor composed of two discrete subcomplexes: one comprised of the previously identified Elp1, Elp2, and Elp3 proteins and another comprised of three novel polypeptides, termed Elp4, Elp5, and Elp6. Disruption of the yeast genes encoding the new Elongator proteins confers phenotypes indistinguishable from those previously described for the other elp mutants, and concomitant disruption of genes encoding proteins in either subcomplex does not confer new phenotypes. Taken together, our results indicate that holo-Elongator is a functional entity in vitro as well as in vivo. Metazoan homologues of Elp1 and Elp3 have previously been reported. We cloned the human homologue of yeast ELP4 and show that this gene is ubiquitously expressed in human tissues.

The form of RNA polymerase II (RNAPII) responsible for transcript elongation is fundamentally different from the form that enters a promoter to form a preinitiation complex (1, 2). During initiation, RNAPII is hypo-phosphorylated and associated with the functionally conserved Mediator complex, a multisubunit factor required for regulation of transcription (3, 4). The association of RNAPII with Mediator and the general transcription factors is severed during promoter clearance, triggered by TFIIH-mediated hyperphosphorylation of the carboxy-terminal repeat domain (CTD) of the largest RNAPII subunit (5–7). During elongation, hyperphosphorylated yeast RNAPII is associated with the Elongator complex. Elongator binds directly to RNAPII, at least partly via the CTD, and the interaction is stabilized by CTD hyperphosphorylation (8).

Elongator was biochemically isolated as a component of elongating RNAPII from salt-stable chromatin but can also be isolated as a discrete, three-subunit complex when starting from the DNA-free soluble fraction of a whole cell extract (8). The genes encoding the Elongator subunits have been identified and shown to play a role in transcription elongation in vivo: ELP1 encodes a protein without discernible motifs (8), whereas Elp2 has multiple WD40 repeats (9), and Elp3 has histone acetyltransferase (HAT) motifs (10). The identification of a highly conserved HAT associated with elongating RNAPII suggests a mechanism for modification and remodeling of chromatin during transcript elongation. Recombinant Elp3 indeed has HAT activity in a gel-based HAT assay, and mutations in the sequence encoding the predicted acetyl-CoA binding pocket of the protein significantly reduce this activity (11). Importantly, when the same point mutations are introduced in yeast, they confer phenotypes that are virtually identical to those resulting from deletion of either of the ELP genes, indicating that the catalytic activity of the Elp3 protein is essential for Elongator function. The in vivo function of Elp3 is overlapping with that of the prototype HAT, Gcn5, the catalytic subunit of SAGA/ADA (12). In the absence of both Elongator and SAGA HAT activity, cells are sick and unable to grow under a large variety of conditions. These phenotypes can be suppressed by concomitantly deleting the genes encoding two histone deacetylases (HDACs), indicating functional redundancy between HDACs as well and supporting the notion that maintenance of a certain overall acetylation level in a cell may be important for cell viability and growth (11). Elongator function, such as HAT activity, is required for normal activation of a number of genes, indicating that Elongator is involved in creating a chromatin structure that is amenable to efficient transcription (8, 10).

To further our understanding of Elongator function, we isolated the complex from extracts of yeast cells in which the gene encoding Elp1 had been modified to express a double affinity-tagged version of the protein. This tag made it possible to rapidly purify Elongator to virtual homogeneity under mild conditions. Here we show that the purified factor is a six-subunit complex, comprised of two discrete, three subunit subcomplexes that easily dissociate. We have identified the three novel proteins of the complex, as well as a human homologue of one of the encoding genes, and provide evidence that holo-Elongator is the functional entity of Elongator in vivo.

**EXPERIMENTAL PROCEDURES**

Expression of Tagged Elp1—A sequence encoding a (His)10-HA epitope tag followed by a transcription termination signal was created by polymerase chain reaction using primers 5′-AGCTGACTAGTCAT-3′ and 5′-CTAGTCTGACTA-GGCGGCGAATTCCGGGCGAATTTCTTATG-3′.

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TACGCGCAAGTCTGACGGCTGG-3' and plasmid pAS2-1 (CLONTech) as a template. The resulting sequence was inserted between the SpeI and SalI sites of pRS304 (13) to yield vector pSE.HISHA-304. Part of the Elp1 open-reading frame was amplified using primers 5'-GCT-ACACTGCCAGAATAATGAGGCTTTTCGCGC-3' and 5'-TGTTGA-CCTAGTAAAAATCAACAATATGACTCTTAGGG-3' and cloned into pSE.HISHA-304 using the XhoI and SpeI sites yielding plasmid pELP1-HISHA-304. After transformation of the yeast strain S. cerevisiae strain BJ2168 (Table I), a trp1Δ clone was isolated in which the 3'-end of the Elp1 gene was replaced, resulting in expression of an Elp1-(His)2-HA fusion protein (Elp1-HisHA; strain JSY549). To ensure that the (His)2-HA epitope tag did not interfere with Elp1 function, the same integration was performed in the W303 strain background, making it possible to do phenotypic analysis and comparison with wild type and elp1Δ cells.

Protein Purification—DNA-free soluble whole cell extract (typically from 0.8–1.0 kg of yeast paste) was prepared from strain JSY549 and subjected to cation-exchange chromatography on Bio-Rex 70 (Bio-Rad) essentially as described previously (8). Protein was stepwise eluted with buffer A (40 mM Hepes-KOH pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 20% (v/v) glycerol) and allowed to bind to 0.4 ml Ni2+-NTA-agarose (Qiagen) at 4 °C overnight with mixing. Bound proteins were then eluted in 1 ml of buffer containing 300 mM KOAc and stored in small aliquots at −80 °C.

Gel filtration was carried out on a SMART chromatography system (Amersham Pharmacia Biotech). A portion of the eluate from the anti-HA immunoprecipitation column was applied via a 25-ml sample loop onto a Superose 6 PC1.6/30 column (Amersham Pharmacia Biotech) collecting 50-μl fractions. Aliquots (1 and 5 μl, respectively) were analyzed by immunoblotting and staining with silver nitrate. Different Elongator (sub)complexes were obtained by anion-exchange chromatography using an AKTA-FPLC (Amersham Pharmacia Biotech). Anti-HA eluate fractions 1–3 were pooled (∼2 ml), diluted with buffer B (25 mM Tris-HAc, pH 7.8, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) to a final concentration of 150 mM KOAc and loaded onto a Mono Q HR5/5 FPLC column (Amersham Pharmacia Biotech). The column was eluted with 2 volumes of buffer B containing 150 mM KOAc and a 10-column volume linear gradient from 150 to 1500 mM KOAc collecting 0.35-ml fractions. Elongator subcomplexes eluted between 1000 and 1450 mM KOAc. Aliquots were analyzed by immunoblotting (1 μl) and staining with silver nitrate (5 μl).

Protein Identification—Gel-fractionated proteins were digested with trypsin, and the mixtures were fractionated on a Poros 50 R2 RP microtip (14). Resulting peptide pools were then analyzed by matrix-assisted laser desorption/ionization reflector time-of-flight (MALDI-TOF) MS using a Reflex III instrument (Bruker Franzen; Bremen, Germany) and by electrospray ionization (ESI) MS/MS on an API 300 triple quadrupole instrument (PE-SCIEX, Thornhill, Canada), modified with an ultrafine ionization source (15). Selected mass values from the MALDI-TOF experiments were taken to search a S. cerevisiae subset of the protein non-redundant data base (NR; NCBI, Bethesda, MD) using the PeptideSearch (16) algorithm. MS/MS spectra were inspected for y ion series to compare with the computer-generated fragment ion series of the predicted tryptic peptides.

Preparation of Antibodies—To produce antibodies recognizing Elp4, Elp5/Iki1, and Elp6, peptides encompassing an amino-terminal cytochrome residue followed by the final carboxyl-terminal 19 amino acids of the corresponding predicted open-reading frames were synthesized. Each of these peptides was coupled via its amino-terminal cysteine residue to Keyhole Limpet Hemocyanin (Calbiochem) by N-terminal benzoyl-N-hydroxysuccinimide ester (Pierce) cross-linking and used to immunize rabbits (Murex). For immunoblotting, the anti-Elp4, -Elp5, and -Elp6 antibodies and their respective preblots were used at a dilution of 1:1000.

Yeast Strains and Phenotypic Analysis—All S. cerevisiae strains used for genetic analysis (Table I) were congenic with strain W303 and grown and manipulated as described previously (8, 10). To analyze killer toxin sensitivity, yeast strains were transformed with plasmid pNW064 encoding the killer toxin ρ-subunit under control of the inducible Gal1-10 promoter (17). Dilutions of the indicated yeast strains were spotted onto SD (ura–trp) medium containing 2% glucose or galactose as indicated.

Expression Analysis of Human Elp4—A full-length human Elp4 cDNA clone was obtained from the NEDO sequencing project (clone KAT08960, GenBank™ accession number AK000505). The entire open-reading frame of human Elp4 was amplified by polymerase chain reaction using primers p123 5'-GAATATCCTCCAGGCGCAATGCGC-3' and p124 5'-GAATATCCTCGAGCGCAATGCGC-3' and radiolabeled with random bevatmers. This probe was hybridized to a human multiple tissue Northern blot according to the manufacturer’s guidelines (CLONTECH). As a control, the blot was probed with a human β-actin cDNA (CLONTECH).

RESULTS

Purification of Elongator from Soluble Whole Cell Extracts—Previously, we purified the Elongator complex from the DNA-free, soluble fraction of a whole cell extract through five to six conventional chromatography steps (8). To facilitate the purification of Elongator and the subsequent analysis of its composition and enzymatic activities, we constructed a haploid S. cerevisiae strain expressing a tagged version of the gene encoding the largest subunit of Elongator, ELPI. The endogenous chromosomal copy was replaced with a gene encoding full-length Elp1 fused to a carboxyl-terminal decahistidine stretch and a HA epitope tag (Fig. 1A). After verification that the tag did not interfere with Elp1 function (data not shown), we purified Elongator using an efficient three-step procedure including two high affinity chromatography steps (Fig. 1B).

First, soluble whole cell extract was loaded onto Bio-Rex 70 cation-exchange resin. Bound proteins were eluted with buffer containing 300 mM, 600 mM, and 1200 mM potassium acetate (KOAc), respectively. Essentially all Elongator was collected in

| Name     | Description                                      | Reference/Source                      |
|----------|--------------------------------------------------|---------------------------------------|
| W303-1A  | MATα, leu2–2, 112, his3–11, 15, trp1–1, ade2–2, can1–100 | Thomas and Rothstein (25)             |
| W303-1B  | MATα, leu2–2, 112, his3–11, 15, trp1–1, ade2–2, can1–100 | Thomas and Rothstein (25)             |
| JYS100   | As W303-1A, but elp1Δ; LEU2                     | Otero et al. (8)                      |
| JYS671   | As W303-1A, but elp1Δ: ADE2                     | This study                            |
| JYS755   | As W303-1A, but elp1Δ: KAN                       | This study                            |
| JYS537   | As W303-1A, but ELPI(His6-HA)::TRP1             | This study                            |
| JYS758   | As W303-1A, but elp1Δ: LEU2                     | This study                            |
| JYS670   | As W303-1B, but elp1Δ: ADE2                     | This study                            |
| JYS784   | As W303-1B, but elp1Δ: KAN                       | This study                            |
| JYS86    | As W303-1B, but elp1Δ: HIS3                      | This study                            |
| JYS757   | As W303-1B, but elp1Δ: LEU2 elp4Δ: ADE2         | This study                            |
| BJ2168   | MATα, prc1–401, pbl1–112, pep3–3, leu2, trp1, ura3–52, gal2 | Jones (26)                            |
| JYS549   | As BJ2168, but ELPI(His6-HA)::TRP1              | This study                            |
The 600 mM KOAc eluate and subsequently applied onto anti-HA immunoaffinity resin. Analysis by immunoblotting showed that virtually all Elp1-HisHA from the Bio-Rex 70 fraction bound to this column and could be eluted by competition with excess peptide containing the HA epitope (Fig. 1C). Interestingly, protein silver staining showed that, in addition to Elp1, Elp2, and Elp3, three additional proteins with apparent molecular weights of 50, 35, and 30 kDa eluted from the immunoaffinity column (Fig. 1D). These three proteins did not bind to the immunoaffinity resin in the absence of tagged Elp1 (data not shown), indicating that they interact specifically with Elp1. Moreover, when the eluates from the anti-HA immunoaffinity resin were subjected to Ni2+-agarose affinity chromatography, the three additional proteins also co-eluted with the previously defined Elongator subunits, providing further evidence that the interaction is specific (Fig. 1D). We designated these putative novel Elongator subunits Elp4, Elp5, and Elp6, respectively.

Identification of Three Novel Elongator Subunits Elp4, Elp5, and Elp6—Peptide mass fingerprinting using MALDI-reTOF mass spectrometry (14, 16, 18) was used to identify the 50-, 35-, and 30-kDa protein bands. We identified the 50-kDa band as the product of the previously defined open-reading frame YPL101W on chromosome XVI (predicted molecular weight Mr 51.2) and named this gene ELP4. The p35/Elp5 protein was identified as the product of open-reading frame YHR187W (chromosome VIII, predicted molecular weight Mr 35.2). Interestingly, this gene was previously identified as the insensitive to killer toxin 1 gene, IKI1, which was identified in the same genetic screen as ELP1/IKI3 and whose inactivation renders yeast cells insensitive to pGKL killer toxin (19). Finally, the 30-kDa protein band was found to correspond to the open-reading frame YMR312W on chromosome XIII (predicted molecular weight Mr 30.6), which we termed ELP6. The predicted molecular weights of the identified open-reading frames correspond well with the apparent molecular masses in all three cases. Analysis of the three amino acid sequences by database searching did not reveal any obvious domain structure or homology to proteins with known function.

To verify the identity of the 50-, 35-, and 30-kDa protein bands, polyclonal rabbit antibodies directed against the carboxy-terminal 19 amino acids of Elp4, Elp5, and Elp6 were generated and tested for reactivity toward purified Elongator by immunoblotting. These antibodies, but not the corresponding preimmune sera, specifically recognized p50/Elp4, p35/Elp5, and p30/Elp6, respectively, in the Elongator preparation, confirming the identification of these proteins as components of the complex (Fig. 2A).

To establish that Elp4, Elp5, and Elp6 are bona fide subunits of Elongator, the purified complex was analyzed by gel filtration chromatography. All six Elongator proteins exactly co-eluted from this resin as judged by protein silver staining and immunoblot analysis using antibodies directed against Elp1, Elp3, Elp4, Elp5, and Elp6, respectively, in the Elongator preparation, confirming the identification of these proteins as components of the complex (Fig. 2A).

Elongator Is Composed of Two Subcomplexes—Previously, we identified Elongator as a three-subunit complex (8). Careful analysis of protein fractions obtained from these earlier purifications showed that proteins with apparent molecular weights corresponding to Elp4, Elp5, and Elp6 eluted from Mono Q slightly later than Elp1, Elp2, and Elp3 (data not shown). We reasoned that Elongator complex might be disrupted by anion-exchange chromatography on Mono Q, or by high salt concentration. Indeed, when affinity-purified six subunit Elongator was loaded onto Mono Q and eluted with increasing salt, three different forms of the complex could be identified (Fig. 3). First, six subunit holo-Elongator eluted at <1100 mM KOAc. Second, the previously identified form of Elongator composed of Elp1, Elp2, and Elp3 (core Elongator) was detected, and, finally, fractions highly enriched in Elp4, Elp5, and Elp6 were obtained. We also observed that Elongator was disrupted in the presence of 2 M NaCl (data not shown). We conclude that Mono Q chromatography and/or high salt concentration can disrupt the Elongator complex, explaining our failure to previously identify the three smallest subunits. These findings indicate that Elongator is composed of two subcomplexes: one comprising the three largest subunits, and

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**Fig. 1.** **Purification of Elongator complex.** A, schematic diagram of tagged Elp1 consisting of full-length Elp1 fused to a decahistidine stretch and an HA epitope tag. B, diagram of the purification scheme. C, immunoblot analysis of fractions from the anti-HA immunoaffinity column. Elp1 protein was detected using rat monoclonal antibody 3F10 (Roche Molecular Biochemicals) recognizing the HA epitope. D, protein staining with silver nitrate of Elongator-containing fractions from the anti-HA immunoaffinity resin and the Ni2+-NTA-agarose column. Elp proteins are indicated. Numbers on the left indicate the positions of the molecular size markers.
the second composed of the three newly identified proteins, Elp4, Elp5, and Elp6.

Disruption of ELP4, ELP5, and ELP6 Genes Results in Typical Elp Phenotypes—To analyze the role of ELP4, ELP5, and ELP6 in vivo, we disrupted one copy of the respective entire open-reading frames in diploid yeast cells, which were subsequently induced to sporulate. After dissection of the resulting tetrads, we noted the appearance of small colonies, which occurred in a 2:2 ratio and co-segregated with the marker used (data not shown). This is reminiscent of the phenotype we previously observed in elp1Δ, elp2Δ, and elp3Δ cells (8–10). The identification of two genes encoding Elongator components, ELP1/IKI3 and ELP5/IKI1, as genes whose inactivation cause insensitivity to pGKL killer toxin (19), prompted us to investigate whether disruption of the remaining four ELP genes also render cells insensitive to the killer toxin derived from the yeast Kluyveromyces lactis. Therefore, we conditionally expressed the y killer toxin subunit intracellularly using a galactose-inducible promoter (17). As shown in Fig. 4A, all elpΔ mutant strains grew normally on medium containing glucose where the killer toxin was not expressed. However, upon expression of killer toxin on galactose-containing medium, wild type cells were unable to grow, whereas all elpΔ mutants were insensitive to killer toxin. The mechanism of killer toxin depends on the histone acetyltransferase activity of Elongator, as inactivation of the complex in an elp3 strain carrying a point mutation in a residue important for the catalytic activity of Elp3 also resulted in insensitivity to killer toxin.

Other phenotypes we observed for elp4Δ, elp5Δ, and elp6Δ cells were an inability to grow at 39 °C (Fig. 4B) and salt sensitivity (Fig. 4C), which were previously observed for elp1Δ, elp2Δ, and elp3Δ mutants (8–10). In addition, elpΔ elp4Δ double mutants displayed phenotypes virtually identical to those of the single mutants, such as growth rate, and sensitivity to elevated temperature and high salt (Fig. 5 and data not shown), as previously observed for all combinations of elp1Δ, elp2Δ, and elp3Δ mutations (9). Taken together, these results indicate that ELP1 through ELP6 are all non-essential genes whose products also form a functional entity in vivo.
Elongator is a labile complex that can be dissociated into its two three-subunit subcomplexes by treatment with high salt or by anionic chromatography. We have identified the genes encoding the three new subunits and shown by genetic analysis that strains lacking any one of these genes have phenotypes that are indistinguishable from those of the previously characterized *elp* strains. Finally, we have cloned the human homologue of yeast ELP4 and shown that it is ubiquitously transcribed.

**Identification of ELP4 Homologues in Higher Eukaryotes**—Further data base searching using the predicted amino acid sequence of ELP4 as a query identified significant homology with several open-reading frames from a variety of higher eukaryotes, including human and mouse (Fig. 6A). No clear domain structure could be identified on the basis of these homologies. Interestingly, however, the putative human and mouse ELP4 proteins are encoded by the human and mouse *PAXNEB* gene, respectively, which is localized on human chromosome 11p13 (mouse chromosome 2), a region implicated with human disease (20). *PAXNEB* is expressed in a variety of human tissues as determined by Northern blot analysis (Fig. 6B) and indicated by the presence of multiple expressed sequence tags (ESTs) derived from different tissues in the data base. The ubiquitous expression pattern suggests a general role for ELP4 in higher eukaryotic cells. In addition, the identification of human and other higher eukaryotic homologues of ELP4 further supports the notion that Elongator is structurally conserved from yeast to man.

**DISCUSSION**

In the present study we isolated Elongator complex by utilizing a rapid purification procedure based largely on affinity chromatography. Our findings can be summarized as follows: Elongator consists of six subunits; the three identified previously, and a novel discrete subcomplex composed of three proteins, termed Elp4, Elp5, and Elp6. We have named the novel factor holo-Elongator, to distinguish it from the core-factor (Elp1, Elp2, and Elp3) that we isolated previously. holo-Elongator is a labile complex that can be dissociated into its two three-subunit subcomplexes by treatment with high salt or by anionic chromatography. We have identified the genes encoding the three new subunits and shown by genetic analysis that strains lacking any one of these genes have phenotypes that are indistinguishable from those of the previously characterized *elp* strains. Finally, we have cloned the human homologue of yeast ELP4 and shown that it is ubiquitously transcribed.

**FIG. 5. Phenotypic analysis of *elp1 elp4* double mutant cells.** A, temperature sensitivity. Wild type, single, and double mutant cells were grown on YPD for 2–4 days at 30 °C, 37 °C, or 39 °C. B, sensitivity to high salt. Serial dilutions of the indicated mutant cells were dropped onto YPD or YPD containing 1 M NaCl and grown for 2 days (YPD) or 3 days (YPD+1 M NaCl).
It may also be significant that a point mutation resulting in loss of Elp3 HAT activity also confers the full elp phenotype. This indicates that the most important function of holo-Elongator lies in its capacity as a HAT.

Elongator As a Putative Target for K. lactis Killer Toxin—

ELP1 is identical to IKI3, and the newly identified ELP5 gene is identical to IKI1. We found that the interesting and intriguing insensitivity to expression of the K. lactis killer/H9253-toxin is a phenotype shared by all the elp strains. While this work was in progress, Frohloff et al. (23) reported the identification of mutants from a killer toxin screen virtually identical to the IKI screen and named their isolated mutants TOT (for toxin target). From this screen, TOT1, 2, and 3 were found to be identical to ELP1, ELP2, and ELP3, respectively. The authors also found that the protein product of IKI1/TOT5, as well as the product of a gene isolated in another killer toxin screen, KTI12/TOT4 (killer toxin insensitive 12), could be co-immunoprecipitated with Elongator proteins, indicating that all the gene products isolated so far as intracellular effectors of the killer toxin interact. Our identification of Iki1/Tot5 as a component of holo-Elongator (Elp5) provides an explanation for the Iki1/Tot5-Elongator interaction observed by Frohloff et al. (23). By contrast, extensive analysis by mass spectrometry did not provide any evidence for the presence of Kti12 in highly purified Elongator preparations. Because the kti12/tot4 mutant was shown to have phenotypes strikingly similar to those of elp mutants (23), however, it is likely that this protein plays a role in Elongator function. Importantly, expression of KTI12/TOT4 from multicopy plasmids, but not similar overexpression of ELP1/TOT1, ELP2/TOT2, ELP3/TOT3, or ELP5/TOT5, confers γ-toxin resistance (23). Taken together, these findings thus suggest that Kti12/Tot4 is not a component of Elongator, but rather influences its activity. This possibility is presently under investigation.

### FIG. 6.

**ELP4** is a ubiquitously expressed gene whose product is conserved in higher eukaryotes. **A**, predicted amino acid sequence of the protein encoded by ELP4 and alignment with sequences from various species using ClustalX (24). **M.m.**, Mus musculus, Q9ER73; **H.s.**, Homo sapiens, Q9NX11; **D.m.**, Drosophila melanogaster, Q9VMQ7; **C.e.**, Caenorhabditis elegans, Q18195; **S.p.**, Schizosaccharomyces pombe, Q9USP1; **S.c.**, S. cerevisiae. Conserved residues are shaded in light gray, identical residues in dark gray, and residues identical in all species are highlighted. Yeast ELP4 is 26% identical/41% similar to human ELP4 (PAXNEB) over the entire sequence. Otherwise, homology between these proteins range from 21% identical and 41% similar (S.c. and D.m.) to 26%/43% (S.c. and S.p.) and 30%/49% (D.m. and H.s.). **B**, expression pattern of human ELP4 (PAXNEB) in the indicated tissues as determined by Northern blot analysis in comparison with the actin control.
Elongator Is Considered among Eukaryotes—Human homologues of ELP1 and ELP3 have been identified by searching the data bases (8, 10). In support of the notion that the structure and function of holo-Elongator is highly conserved among eukaryotes, we identified a human homologue of the yeast ELP4 gene, which has previously been submitted to the data bases and named PAXNEB. This gene is ubiquitously expressed, and is located in a region on chromosome 11 that has been implicated in human disease. Heterozygous deletion of the 11p13 region gives rise to WAGR syndrome: Wilm’s tumor, Aniridia, Genitourinary abnormalities, and mental Retardation. Most of these abnormalities are due to deletion of the well studied disease genes, PAX6 and WT1, but the cause(s) of the mental retardation remains to be identified. Other disease-related loci, such as those associated with loss of heterozygosity in breast and bladder cancers, also map to this region (Ref. 20 and references therein). We are presently isolating human Elongator with the aim to explore the molecular structure and function of Elongator in metazoans.

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REFERENCES
1. Dahmus, M. E. (1996) J. Biol. Chem. 271, 19009–19012
2. Svejstrup, J. Q., Vichi, P., and Egly, J. M. (1996) Trends Biochem. Sci. 21, 346–3430
3. Malik, S., and Roeder, R. G. (2000) J. Biol. Chem. 275, 225–229
4. Otero, G., Fellows, J., Li, Y., de Bizemont, T., Dirac, A. M. G., Gustafsson, C. M., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J. Q. (1999) Mol. Cell. 3, 109–118
5. Wittschieben, B. O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C. D., Tempst, P., and Svejstrup, J. Q. (1999) Mol. Cell. 4, 123–128
6. Wittschieben, B. O., Fellows, J., Du, W., Stillman, D. J., and Svejstrup, J. Q. (2000) EMBO Journal 19, 3060–3068
7. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997) Genes Dev. 11, 1640–1650
8. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
9. Myers, L. C., and Kornberg, R. D. (2000) EMBO J. 19, 3060–3068
10. Caires, D. R., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (1998) Mol. Cell 2, 639–651
11. Frohloff, F., Fichtner, L., Jablonowski, D., Breunig, K. D., and Scharffrath, R. (2001) EMBO J. 20, 2663–2673
12. Thomas, B. J., and Rothstein, R. (1989) Cell 56, 619–630
13. Jones, E. W. (1977) J. Chromatogr. A. 156, 704–709
14. Thomas, B. J., and Rothstein, R. (1989) Cell. Biosci. Biotechnol. Biochem. 61, 704–709
15. Yudkovsky, N., Ranish, J. A., and Hahn, S. (2000) Nature 408, 225–229
