Identification and Characterization of Elongin A2, a New Member of the Elongin Family of Transcription Elongation Factors, Specifically Expressed in the Testis*

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The Elongin complex stimulates the rate of transcription elongation by RNA polymerase II by suppressing the transient pausing of the polymerase at many sites along the DNA template. Elongin is composed of a transcriptionally active A subunit and two small regulatory B and C subunits, the latter of which bind stably to each other to form a binary complex that interacts with Elongin A and strongly induces its transcriptional activity. To further understand the roles of Elongin in transcriptional regulation, we attempted to identify Elongin-related proteins. Here, we report on the cloning, expression, and characterization of human Elongin A2, a novel transcription elongation factor that exhibited 47% identity and 61% similarity to Elongin A. Biochemical studies have shown that Elongin A2 stimulates the rate of transcription elongation by RNA polymerase II and is capable of forming a stable complex with Elongin BC. However, in contrast to Elongin A, its transcriptional activity is not activated by Elongin BC. Northern blot analysis revealed that Elongin A2 mRNA was specifically expressed in the testis, suggesting that Elongin A2 may regulate the transcription of testis-specific genes.

Eukaryotic messenger RNA synthesis is a complex biochemical process controlled by the concerted action of a set of general transcription factors that regulate the activity of RNA polymerase II during the initiation and elongation stages of transcription. At least six general transcription initiation factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIF) have been identified in eukaryotic cells and found to promote selective binding of RNA polymerase II to promoters and to support a basal level of transcription (1). In addition to the general initiation factors, six general elongation factors (SII, P-TEF-b, TFIIF, Elongin, eleven-nineteen lysine-rich leukemia protein, and the Cockayne syndrome complementation group B protein) have been defined biochemically and found to increase the efficiency of transcription elongation by RNA polymerase II (2–4). Of the general elongation factors, SII and P-TEFb prevent RNA polymerase II from prematurely arresting transcription. All of the rest of the elongation factors, TFIIF, Elongin, eleven-nineteen lysine-rich leukemia protein, and Cockayne syndrome complementation group B protein, act to increase the overall rate of RNA chain elongation by RNA polymerase II by suppressing the transient pausing of polymerase at many sites along the DNA template.

Elongin was initially identified as a heterotrimer composed of A, B, and C subunits of ~770, 118, and 112 amino acids, respectively (5–8). Elongin A is the transcriptionally active component of the Elongin complex, whereas Elongins B and C are positive regulatory subunits. Biochemical studies have shown that Elongins B and C form a stable Elongin BC complex that binds to Elongin A and strongly induces its transcriptional activity (8, 9). Elongin C functions as the inducing ligand and activates transcription through interaction with a short conserved motif (consensus sequence LXXXXXXXVL/I) in the elongation activation domain of Elongin A (9). Elongin B, a member of the ubiquitin homology gene family, appears to play a chaperone-like role in the assembly of the Elongin complex by binding to Elongin C and facilitating its interaction with Elongin A (7, 8). Notably, Elongins B and C are also found as integral components of a multiassembly complex containing the product of the von Hippel-Lindau (VHL) tumor suppressor gene (10, 11). Elongin A and the VHL protein share the Elongin BC binding site motif, and consistent with the assumption of a role for the Elongin BC in tumor suppression, more than 70% of VHL mutations found in VHL families and sporadic clear cell renal carcinomas are associated with mutation or deletion of this site; in all the cases tested, these mutant VHL proteins exhibited substantially reduced binding to Elongin BC (10, 12).

As part of our effort to understand the function, mechanism of action, and regulation of the Elongin complex, we attempted to identify homologs of the Elongin subunits. In this report, we describe the identification and characterization of Elongin A2, a novel Elongin A-related transcription elongation factor from human cells.

EXPERIMENTAL PROCEDURES

Materials—Unlabeled ultrapure ribonucleoside 5'-triphosphate and [α-32P]CTP (>400 Ci/mmol) were purchased from Amersham Pharmacia Biotech. Bovine serum albumin, polyvinyl alcohol type II, isopropyl...
β-d-thigalactoside, phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, and benzamidine were obtained from Sigma, placental ribonuclease inhibitor was from Promega, and Ni²⁺-nitritotriacetic acid agarose (Ni²⁺-agarose) was from Qiagen.

Isolation of Human Elongin A2 cDNA—The data base of human expressed sequence tags was searched with the human Elongin A2 cDNA sequence (13), and a 420-base pair sequence (accession number AA861139), part of which had exhibited homology to the 3'-end of the coding region of the Elongin A cDNA, was found in a human testis expressed sequence tag data base. This 420-base pair cDNA fragment was used as a probe to screen a plasmid pλPshe human testis cDNA library (TAKARA), and one positive cDNA clone was isolated. The nucleotide sequence of the clone was 263–753 of human Elongin A2 was obtained. The human Elongin A2 partial cDNA sequences enabled us to design a gene-specific primer, 5'-CTTGTCGAGTGTGTCGAGGC-3', which was used to obtain the 5'-end of human Elongin A2 by rapid amplification of the cDNA ends (RACE) with Marathon-Ready testis cDNA as the template (CLONTECH). The resulting 980-base pair 5'-RACE product was cloned into pGEM-T Easy (Promega). Full-length human Elongin A2 cDNA was obtained by overlap extension (14) using KOD DNA polymerase (TOYOBO).

DNA Sequencing and Northern Blot Analysis—DNA sequencing was performed by using an automated sequencer (ABI Prism 310, Applied Biosystems). Human multiple tissue Northern blots I and II (CLONTECH) were performed by using an automated sequencer (ABI Prism 310, Applied Biosystems). Human multiple tissue Northern blots I and II (CLONTECH) were probed with 960-base pair 5'-labelled cDNA, which was used to obtain partial cDNA sequences enabled us to design a gene-specific primer, 5'-GATCCTTATGCAGCGAGGTTCATACACGG-3' (Biosearch Technologies, Inc.) supplemented with 10% bovine serum albumin (Sigma), and 0.1% SDS. The column was eluted at 1 ml/min with a 50-ml gradient from 0.1 to 0.8 M KCl in the same buffer. Recombinant Elongin A2 was eluted at 0.45 and 0.5 M KCl. Recombinant Elongin A, B, and C subunits were expressed in E. coli and purified as described previously (9).

Expression of Recombinant Proteins in Insect Cells—cDNAs encoding human Elongin A2 and rat Elongin A containing 10-histidine and FLAG tags at their N-terminal were cloned into the Ncol-BamHI site of the pBacPAK-His1 vector (CLONTECH). cDNAs encoding untagged rat Elongin B and rat Elongin C were cloned into the Xhol-BglII site of the pBacPAK8 vector (CLONTECH). Spodoptera frugiperda (SF9) cells were cultured as monolayers in Grace's insect medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (JRH Biosciences) at 27°C. Recombinant transfer plasmid containing each Elongin subunit was cotransfected with Bsu36I-digested BacPAK6 virus DNA (CLONTECH) into SF9 cells by Lipofectin (LifeTechnologies, Inc.)—mediated transfection, and the resultant viral pool was collected four days later and amplified three times. For expression of the recombinant proteins, SF9 cells were seeded at a density of 2 × 10⁶ cells/175-cm² flask and infected with recombinant baculoviruses expressing 10-histidine-tagged Elongin A2 or Elongin A either with or without baculoviruses expressing untagged Elongin B and C subunits at a multiplicity of infection of 10–20. 60–72 h after infection, the cells were gently dislodged from culture flasks and collected by centrifugation at 1000 × g at 4°C and then resuspended in 10 ml of ice-cold lysis buffer (20 mM Tris-HCl, 0.1% Nonidet P-40, 1 mM dithiothreitol, and 0.5 mM PMSF) and lysed by brief sonication. The cell lysates were centrifuged at 100,000 × g for 60 min. Recombinant Elongin A2 and C were purified from the supernatants using Ni²⁺-agarose affinity chromatography. 5 ml of each supernatant was applied to a 1-ml Ni²⁺-agarose column pre-equilibrated in buffer A containing 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 0.1% Nonidet P-40, 1 mM dithiothreitol. The cell pellets were resuspended in 5 ml of lysis buffer (20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 0.1% Nonidet P-40, 0.5 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml aprotinin, and 1 mM benzamidine) and lysed by brief sonication. The cell lysates were then clarified by centrifugation for 20 min at 100,000 × g to remove insoluble materials. Recombinant Elongin A2 was purified from the supernatants using Ni²⁺-agarose-10 histidine column (Clontech). Recombinant Elongin A2 was subjected to SDS-PAGE, stained with Coomassie Brilliant Blue G-250, and visualized by autoradiography.
ruses expressing human Elongin A2 or rat Elongin A with both 10-
histidine and FLAG epitope tags at their NH2 termini were infected into
Sf9 cells either with or without baculoviruses expressing untagged rat
Elongins B and C. The cells were harvested 60–72 h postinfection,
resuspended in lysis buffer (20 mM Tris-HCl (pH 7.9), 500 mM NaCl,
0.1% Nonidet P-40, 0.5 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml aprotinin,
and 1 mM benzamidine), and lysed by brief sonication. The cell lysates
were incubated for 2 h at 4 °C with 100 μl of Ni2+ -agarose pre-
equilibrated in lysis buffer containing 20 mM imidazole (pH 7.9) and
then centrifuged for 1 min at 2000 rpm. Following centrifugation, the
supernatants containing the unbound proteins were collected, and the
Ni2+ -agarose was washed four times by resuspension in 1 ml of lysis
buffer containing 20 mM imidazole (pH 7.9) and centrifuged for 1 min at
2000 rpm. Finally, bound proteins were eluted with 200 μl of lysis
buffer containing 300 mM imidazole (pH 7.9). Aliquots of each fraction
were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE),
transferred to a polyvinylidene difluoride membrane (Millipore), and
probed with the appropriate antibody.

RESULTS

Isolation of cDNA Clone for Human Elongin A2—To study
the possible existence of a novel member of the Elongin protein
family, a data base of human expressed sequence tags was
searched with the human Elongin A sequence (13) as the query
sequence. One expressed sequence tag clone (accession number
AA861139) was found in a testis data base that showed 50%
identity to the 3'-end of the coding region of human Elongin A
at the amino acid level. An insert of this clone was used to
screen a human testis cDNA library, and one cDNA clone
containing the 1.8-kb insert was obtained. The 5'-end of cDNA
was obtained by 5'-RACE analysis with pooled human testis
cDNAs. Full-length Elongin A2 cDNA was obtained by overlap
extension (14) using these two cDNA fragments as templates.
The cDNA clone covered 114 nucleotides of the 5'-untrans-
lated sequence, the whole coding region, and 328 nucleotides of
the 3'-untranslated sequence. The nucleotides flanking the
start methionine conformed to the Kozak consensus sequence
(18). An in-frame stop codon was located 111 nucleotides up-
stream from the start ATG. The 3'-untranslated sequence con-
tained the putative polyadenylation signal TATAAA, which is

accompanied 12 base pairs downstream by a poly (A)+ tail of 20
nucleotides (these cDNA sequences are not shown but have
been deposited in the GenBankTM data base).

Amino Acid Sequence of Human Elongin A2 and Its Compar-
ison with That of Human Elongin A—The Elongin A2 cDNA
contained an open reading frame encoding a protein of 753
amino acids with a calculated molecular mass of 83,932 Da
(Fig. 1). As determined by the MegAlign program of the Laser-
gene (Madison, WI) system, Elongin A2 is 47% identical and
61% similar to human Elongin A. Although Elongin A2 has no
obvious structural motifs such as zinc finger, leucine zipper, or
helix-turn-helix motifs commonly found in transcription fac-
tors, comparison between Elongin A2 and Elongin A revealed
two conserved structural features. The first was the SII simi-
larity domain (motif I) present at the NH2 terminus. The two

FIG. 1. Comparison of the deduced amino acid sequences between human Elongin A2 and Elongin A. Identical amino acids are
shown in white letters on a black background and chemically similar amino acids are shown in black letters on a gray background. Motifs I and
II are indicated by single and double underlines, respectively. Numbers indicate amino acid positions in each protein. EloA2, Elongin A2; EloA,
Elongin A.

FIG. 2. Expression of Elongin A2 and Elongin A mRNA in hu-
man tissue. Multiple tissue Northern blots (CLONTECH), containing
2 μg of poly(A)+ RNAs of the indicated human tissues, were hybridized
with an Elongin A2 (upper), Elongin A (middle), or β-actin cDNA probe
(lower). The size of the RNA standards is indicated on the left. PBL,
peripheral blood leukocyte; EloA2, Elongin A2; EloA, Elongin A.
The proteins exhibited 64% identity over a 107-amino acid overlap. The other was the Elongin BC binding sequence (motif II) present at the COOH terminus of these proteins. This ~10-amino acid-consensus sequence (T/S)(LXXXXXXX(V/L/I)) has been found not only in Elongin A, but also in VHL, and the suppressor of cytokine signaling family of proteins (8, 9, 19).

Expression of Elongin A2 and Elongin A in Various Human Tissues—To examine the tissue distribution of Elongin A2 and Elongin A expression, Northern blots containing poly(A)+ RNA from various human tissues were hybridized with Elongin A2- and Elongin A-specific probes. As shown in Fig. 2, only the RNA from the testis showed a single band of ~3.0 kb hybridizing with the human Elongin A2 probe. On the other hand, the Elongin A probe hybridized to two mRNA species of ~5.2 and ~2.8 kb. Although the intensity of the signals exhibited tissue-to-tissue variation, these two mRNA species were detected in all of the tissues examined. It remains to be determined whether the ~5.2 and ~2.8 kb mRNAs were alternatively spliced forms of Elongin A or a cross-hybridized form from a closely related gene.

Chromosomal Location of Human Elongin A2 Gene—The chromosomal location of human Elongin A2 gene was determined by fluorescence in situ hybridization as described under “Experimental Procedures.” Metaphase chromosomes with replication bands were hybridized with a biotinylated human Elongin A2 cDNA probe. The hybridized signals were located on the q21.1 band of human chromosome 18, and no accumulation of signals was observed on any other chromosomes (Fig. 3).

Elongin A2 Stimulates Transcription Elongation—In a previous study, we demonstrated that Elongin A is capable of stimulating the rate of RNA chain elongation by RNA polymerase II (8). To investigate whether Elongin A2 is also capable of stimulating transcription elongation by RNA polymerase II, a DNA fragment containing the open reading frame of Elongin A2 was introduced into both E. coli and baculovirus expression vectors. Recombinant Elongin A2 with an NH2-terminal 10-histidine tag was expressed in E. coli and purified from guanidine-solubilized inclusion bodies by sequential Ni2+-agarose and TSK SP-5PW chromatography. Recombinant Elongin A2 with 10-histidine and FLAG tags at the NH2 terminus was expressed in insect cells and purified from the soluble fraction of the cell lysates by Ni2+-agarose chromatography. Recombinant Elongin A2 in both preparations had an apparent molecular mass of 100 kDa and a slightly faster electrophoretic mobility than Elongin A (Fig. 4).

To examine the ability of Elongin A2 to stimulate the rate of RNA chain elongation by RNA polymerase II and to compare its activity with that of Elongin A, an oligo(dC)-tailed template assay was performed. In the experiment shown in Fig. 5, transcription was initiated by the addition of RNA polymerase II to reaction mixtures containing the pCpGR220S/P/X template and ATP, GTP, and [α-32P]CTP. After a 20-min incubation, transcripts of ~135 nucleotides were synthesized on the T-less cassette of the template (left panel, lanes 1). These transcripts were then chased with a limiting concentration of UTP and an excess of nonradioactive CTP in the presence or absence of equivalent amounts of baculovirus-expressed recombinant Elongin A2 or Elongin A. In the absence of Elongin, a substantial portion of the ~135-nucleotide transcripts persisted for at least 7 min after the addition of UTP and nonradioactive CTP (left panel, lane 1). In the presence of increasing concentrations of either Elongin A2 or Elongin A (left panel, lanes 2–7), nearly all of the ~135-nucleotide transcripts had been chased into longer transcripts. Notably, the transcripts synthesized in the presence of Elongin A were substantially longer than those synthesized in the presence of Elongin A2. When we used E. coli-expressed Elongin A2 and Elongin A, almost identical results were obtained (data not shown). Thus, Elongin A2 is capable of stimulating RNA chain elongation by RNA polymerase II in the absence of other transcription factors, although it appears to be less active than Elongin A.

These findings were confirmed by an experiment in which we measured the kinetics of accumulation of the long transcripts and of the distribution of RNA intermediates in the presence or absence of equivalent amounts of Elongin A2 or Elongin A (Fig. 5, right panel). In the absence of Elongin, nearly all of the ~135-nucleotide transcripts had been chased into longer tran-
scripts ~20 min after the addition of UTP (lanes 2–5). With the addition of Elongin A2, RNA chain elongation progressed at an intermediate rate, with nearly all of the ~135-nucleotide transcripts disappearing within 10 min (lanes 6–9). With the addition of Elongin A, the elongation progressed more rapidly, with nearly all of the ~135-nucleotide transcripts disappearing within 5 min (lanes 10–13).

**Elongin A2 Forms a Stable Complex with Elongin BC**—Because of the high degree of conservation of the putative Elongin BC binding residues in Elongin A2, we expected that Elongin A2 would also interact with Elongins B and C. To assess the ability of Elongin A2 to bind with Elongins B and C to form an Elongin A2-BC complex, we assayed human Elongin A2 containing 10-histidine and FLAG tags at the NH2 terminus for its ability to retain the untagged Elongins B and C on Ni2+-agarose. In this experiment, Elongin A2 was coexpressed with Elongins B and C in Sf9 cells, and cell lysates were subjected to Ni2+-agarose chromatography. Unbound and bound protein fractions were collected, and equivalent amounts of each fraction were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting. As shown in Fig. 6, untagged Elongins B and C did not bind to Ni2+-agarose (lanes 1–3), but were retained on the column in the presence of 10-histidine-tagged Elongin A2 (lanes 7–9). The amounts of Elongins B and C retained on the column were approximately equivalent to those retained by Elongin A (lanes 13–15). Thus, as predicted by the sequence homology, Elongin A2 can also bind to Elongin BC as strongly as Elongin A.

**Transcriptional Activity of Elongin A2 Is Not Activated by Elongin BC**—In a previous study, we demonstrated that the transcriptional activity of Elongin A is strongly activated by Elongin BC (8, 9). To assess the contribution of Elongin BC to the transcriptional activity of Elongin A2, purified Elongin BC complex at increasing concentrations was preincubated at 4 °C for 30 min in the absence or presence of purified Elongin A2 or Elongin A and their activities were then measured using the oligo(dC)-tailed template assay (Fig. 7, left panel). As we reported previously, purified Elongin BC complex by itself had no detectable effect on the rate of RNA chain elongation (lanes 1–3), and the activity of Elongin A on RNA chain elongation was strongly activated by Elongin BC (lanes 7–9). However, the addition of the increasing concentrations of Elongin BC had no detectable effect on the activity of Elongin A2.

We considered a possible explanation for the observed unresponsiveness of Elongin A2 to Elongin BC. First, it is possible that Elongin A2 purified from insect cells is associated with endogenous insect cell Elongin BC. To rule out this possibility, we performed an oligo(dC)-tailed template assay using E. coli-expressed Elongin A2 (Fig. 7, right panel). The activity of Elongin A2 purified from E. coli was not activated by Elongin BC (lanes 5–7), suggesting that the lack of stimulation of Elongin A2 by the Elongin BC complex was not due to contaminating insect cell Elongin BC in our Elongin A2 preparations. Second, it is also possible that Elongin A2 may not be able to fold properly in the absence of Elongin BC, and coexpression of Elongin A2 and Elongins B and C is essential for the assembly of functional heterotrimers. We therefore infected baculoviruses expressing Elongin A2 to Elongin BC. To rule out this possibility, we performed an oligo(dC)-tailed template assay using E. coli-expressed Elongin A2 (Fig. 7, right panel). The activity of Elongin A2 purified from E. coli was not activated by Elongin BC (lanes 5–7), suggesting that the lack of stimulation of Elongin A2 by the Elongin BC complex was not due to contaminating insect cell Elongin BC in our Elongin A2 preparations. Second, it is also possible that Elongin A2 may not be able to fold properly in the absence of Elongin BC, and coexpression of Elongin A2 and Elongins B and C is essential for the assembly of functional heterotrimers. We therefore infected baculoviruses expressing Elongin A2 in the presence or absence of baculoviruses expressing Elongins B and C in insect cells and purified the Elongin A2-BC complex or Elongin A2 using Ni2+-agarose chromatography. We then compared the specific activity of these proteins. However, no detectable difference was observed (data not shown), suggesting that coexpression is not important for the responsiveness of Elongin A2 to Elongin BC.

**DISCUSSION**

In this report, we describe the isolation and characterization of a human cDNA encoding Elongin A2, a novel RNA polymerase II elongation factor that is specifically expressed in the testis. Elongin A2 is a 753-amino acid protein exhibiting 47% identity and 61% similarity to the previously characterized transcription elongation factor, Elongin A (8, 13). Like Elongin A, Elongin A2 is able to stimulate the rate of elongation by RNA polymerase II in the oligo(dC)-tailed template assay,
suggesting that it stimulates elongation through a direct interaction with RNA polymerase II, template DNA, the nascent transcript, or some combination of these components of the ternary RNA polymerase II elongation complex.

We note that the SII similarity region (motif I) at the NH$_2$ terminus of Elongin A is highly conserved in Elongin A2. The previous results indicate that the NH$_2$ termini of both Elongin A and SII are dispensable for the elongation-stimulatory activities of these proteins in vitro and for the rescue of the phenotype of an SII deletion mutant in the yeast (20–22). The findings that the NH$_2$ termini of both Elongin A and SII are capable of binding to RNA polymerase II holoenzyme (23), however, suggests that this region may play an in vivo regulatory role not yet revealed by current assays.

Elongin A2 also contains the Elongin BC binding sequence (motif II), consensus (T/S)LXXXCXXX(V/I), at the COOH terminus. Our previous results indicate that this sequence motif is important for the induction of the transcriptional activity of Elongin A by Elongin BC (9). Consistent with the conservation of this sequence motif, Elongin A2 was shown to be able to form a stable complex with Elongin BC. In contrast to Elongin A, however, the transcriptional activity of Elongin A2 was not activated by Elongin BC. What is the reason for the unresponsiveness of Elongin A2? Although the mechanism of induction of Elongin A by the Elongin BC complex has not yet been established at the molecular level, binding of Elongin BC could cause the elongation activation domain of Elongin A to adopt a more transcriptionally active conformation. If Elongin A activity were solely induced by this allosteric mechanism, it is possible that the elongation activation domain of Elongin A2 might be less flexible than that of Elongin A and resistant to conformational change. Indeed, although the sequence constituting the elongation activation domain of Elongin A, residues 520–690, is well conserved in Elongin A2, they do not share

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**Fig. 6.** Elongin A2 forms a complex with Elongins B and C. Insect cells infected with the recombinant baculoviruses expressing the indicated combinations of the proteins were lysed, and the proteins were precipitated with Ni$_2^+$-agarose resin. Load, unbound, and bound fractions were subjected to SDS-PAGE followed by Western blotting using anti-FLAG (upper), anti-Elongin B (middle), or anti-Elongin C (lower) antibody.

**Fig. 7.** Effect of Elongin BC on the transcriptional elongation activity of Elongin A2 and Elongin A. Left panel, the oligo(dC)-tailed template assays were performed in the absence (lanes 1–3) or presence of 3 nM Elongin A2 (lanes 4–6), or 3 nM Elongin A (lanes 7–9) purified from insect cells. Elongin BC was present in reaction mixtures at 1.5 nM (lanes 2, 5, and 8) or 3 nM (lanes 3, 6, and 9). Right panel, The oligo(dC)-tailed template assays were performed in the absence (lane 1) or presence of 3 nM Elongin A (lanes 2–4) or 3 nM Elongin A2 (lanes 5–7) purified from E. coli. Elongin BC was present in reaction mixtures at 1.5 nM (lanes 3 and 6) or 3 nM (lanes 4 and 7).
sequence similarity throughout this region. The other possibility is that Elongin A2 might have its own partners in vivo, as noted for the TATA-binding protein-related factor (24, 25). The Drosophila TATA-binding protein-related factor is expressed only in the central nervous system and the testis and is complexed in vivo with its own set of associated factors (designated neuronal TATA-binding protein-associated factors) apparently distinct from the TATA-binding protein-associated factors (24, 25). The localization of the TATA-binding protein-related factor to a small number of specific loci on Drosophila polytene chromosomes suggests that the TATA-binding protein-related factor-neuronal TATA-binding protein-associated factor complex supports transcription from a subset of tissue-specific genes. A similar situation might exist for Elongin A2, and it is possible that the activity of Elongin A2 is only regulated by factors, such as testis-specific homologs of Elongins B and C.

What might be the function of Elongin A2 in vivo? Because Elongin A, TFII F, and eleven-nineteen lysine-rich leukemia protein are capable of stimulating the rate of elongation through a wide variety of DNA templates tested in vitro, these factors have been considered to be general elongation factors. If this were also the case with Elongin A2, this factor could function as a testis-specific general elongation factor and work cooperatively with Elongin A to support the relatively high level of transcription in this tissue. Alternatively, Elongin A2 could regulate the expression of only a subset of genes. The discovery that DNA binding transcriptional activators, such as VP-16, E1A, and p53, which regulate transcription in part by stimulating the rate of elongation, raises the possibility that transcription elongation factors including Elongin A2 could be targets for gene-specific transcriptional activators in vivo and are recruited to the promoter regions of specific genes (26, 27). Furthermore, although Elongin A2 did not exert any detectable inhibition of Elongin A activity in the in vitro assay (data not shown), it is also possible that Elongin A2 works as a negative regulator of Elongin A in vivo by binding to Elongin BC and inhibiting its ability to activate the transcriptional activity of Elongin A in the testis. Future biochemical characterization of these proteins that associate with Elongin A2, as well as identification of potential Elongin A2-responsive genes will undoubtedly clarify this issue.

Finally, the testis-specific expression of Elongin A2 together with the overexpression of RNA polymerase II and several transcription initiation factors in this tissue suggests that Elongin A2 plays some important role in spermatogenesis (28, 29). Because expression of Elongin A2 is not detectable in the ovary, Elongin A2 does not appear to play any general role in meiosis. Determination of the cell type specificity of Elongin A2 expression and generation of Elongin A2-deficient mice will be required to gain insight into the function of Elongin A2 in vivo.

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Identification and Characterization of Elongin A2, a New Member of the Elongin Family of Transcription Elongation Factors, Specifically Expressed in the Testis
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