Human Elongator complex was purified to virtual homogeneity from HeLa cell extracts. The purified factor can exist in two forms: a six-subunit complex, holo-Elongator, which has histone acetyltransferase activity directed against histone H3 and H4, and a three-subunit core form, which does not have histone acetyltransferase activity despite containing the catalytic Elp3 subunit. Elongator is a component of early elongation complexes formed in HeLa nuclear extracts and can interact directly with RNA polymerase II in solution. Several human homologues of the yeast Elongator subunits were identified as subunits of the human Elongator complex, including StIP1 (STAT-interacting protein 1) and IKAP (IKK complex-associated protein). Mutations in IKAP can result in the severe human disorder familial dysautonomia, raising the possibility that this disease might be due to compromised Elongator function and therefore could be a transcription disorder.

RNA polymerase II (RNAPII)\(^1\) transcription can be reconstituted \textit{in vitro} with a minimal set of general transcription factors, such as TBP, TFIIB, TFIIF, TFIIE, and TFIH (1, 2). Each of these factors is absolutely required for promoter-specific initiation of transcription under most conditions. By contrast, transcript elongation by RNAPII can occur in the absence of initiation of transcription under most conditions. By contrast, over the last few years a plethora in vitro evidence has accumulated that specifically associate with the elongating form of RNAPII (4). Purification of chromatin-associated, hyperphosphorylated RNAPII from yeast led to the isolation of the Elongator complex and identification of the genes (\textit{ELP}1–\textit{ELP}6) encoding subunits of this multisubunit factor (4–7). The functional entity of Elongator complex has recently been shown to be an unstable six-subunit complex, termed holo-Elongator, which can dissociate into two discrete three-subunit subcomplexes upon treatment with high salt and/or MonoQ chromatography (7). One of these subcomplexes is the Elp3-containing core Elongator complex initially identified (4), and the other is a complex of the Elp4, Elp5, and Elp6 proteins (7). Yeast cells lacking the \textit{ELP} genes are viable but display a variety of phenotypes consistent with a role for the factor in transcript elongation \textit{in vivo}. Significantly, the Elp3 subunit is a highly conserved histone acetyltransferase (HAT) (6). Mutations that debilitate the HAT activity of Elp3 \textit{in vitro} also confer \textit{elp} phenotypes \textit{in vivo}, indicating that the HAT activity of Elongator is required for its function (7, 8).

Homologues of several Elongator subunits, such as the WD40 repeat protein Elp2 (5), the HAT Elp3 (6), and Elp1 and Elp4 can be found by data base searching in the genomes of higher eukaryotic cells. Intriguingly, the closest homologue of Elp1 in human cells is encoded by the IKAP gene (4, 9), while the closest mouse homologue of Elp2 is StIP1 (10). Mutations in the IKAP gene can result in the severe human hereditary disorder familial dysautonomia (11, 12), while StIP1 was identified through its interaction with the transcriptional activator signal transducer and activator of transcription 3 (STAT3) (10).

Here, we report the purification and characterization of the human Elongator complex. Our results reveal extensive structural and functional similarity between the yeast and the human complex. Most notably, human holo-Elongator is a histone acetyltransferase complex, which in addition to human ELP3 (hELP3) contains IKAP, human StIP1, human ELP4 (hELP4), and two additional proteins as subunits. The identification of IKAP as an Elongator subunit suggests a connection between Elongator and human disease.

**EXPERIMENTAL PROCEDURES**

Buffers and Solutions—Buffers A, B, and C were as described previously (13), except that KCl was used as salt in buffers A and B. Buffer D was 40 mM Tris-HCl, pH 7.5, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 0.05% Nonidet P-40, and the concentration of KCl indicated after the hyphen. Protease inhibitors were included in all buffers used (14).

**Purification of Elongator from HeLa Cells**—Throughout purification, Elongator was followed by Western blotting using anti-IKAP (15) and
anti-Elp3 (4) antibodies. HeLa cell extract was prepared essentially as described (16) until after the ultracentrifugation step. The soluble cell extract was then dialyzed into buffer B-50 (50 mM KCl), loaded onto heparin-Sepharose (Amersham Biosciences, Inc.), and eluted stepwise with buffer B-150, B-300, B-450, and B-700. Proteins from the B-300 eluate were loaded, dialyzed into buffer B-50, loaded onto MonoS HR 10/10 column (Amersham Biosciences), and eluted with a linear gradient from 70 to 800 mM salt in buffer B-150, and 1-ml fractions were collected. The Elongator-containing fractions were diluted, applied to Progel-TSK DEAE-5PW (Supelco) equilibrated in buffer C-10, and eluted with a gradient from 10 to 400 mM potassium phosphate over 10 column volumes (CV) in buffer C. Elongator-containing fractions were dialed into buffer A-50, loaded onto a MonoS HR 10/10 column (Amersham Biosciences), and eluted with a linear gradient from 70 to 800 mM salt in buffer A over 15 CV. Fractions containing Elongator were pooled, dialyzed into buffer B-50, loaded onto MonoS HR 5/5 column (Amersham Biosciences) equilibrated in buffer B-50, and eluted with a linear gradient from 70 to 500 mM salt in buffer B over 10 CV. Elongator fractions were loaded onto a Sephacryl S-300 (26/20) gel filtration column (Amersham Biosciences), equilibrated in buffer B-150, and 1-ml fractions were collected. The Elongator-containing fractions were diluted, applied to Progel-TSK DEAE-5PF (Supelco) equilibrated in buffer B-50, and eluted with a linear gradient from 70 to 800 mM salt in buffer B over 10 CV. Elongator fractions were loaded onto HTP hydroxypatite (Bio-Rad), and the resin was resolved as described above. The purity of the fractions from hydroxypatite was determined by silver staining.

In addition to some of the steps described, the procedure to purify human Elp3 from insect cells (DEAE-Sepharose fast flow (Amersham Biosciences), Inc.), with proteins eluted stepwise in buffer B containing 150, 300, 450, and 700 mM salt. Proteins in the B-300 eluate were precipitated by ammonium sulfate precipitation (40% saturation) before dialysis into buffer A-50 and MonoS HR 5/5 chromatography as described above. Elongator fractions were incubated with IKAP antibody resin for several hours to overnight at 4 °C with gentle mixing. Following washing with B-100, antibody-associated proteins were released by batch incubation with 1 CV of 1 mg/ml IKAP peptide (15) in buffer D-500 at 4 °C for several hours and eluted from the resin by gravity flow.

**Protein Identification**—Gel-fractionated proteins were digested with trypsin, and peptides were analyzed by matrix-assisted laser desorption/ionization reflectron time-of-flight MS and by electrospray ionization MS/MS as previously described (7). Selected mass values from the matrix-assisted laser desorption/ionization time-of-flight experiments were taken to search the protein nonredundant data base (NCBI, Bethesda, MD) using the PeptideSearch (17) algorithm. MS/MS spectra were inspected for y' ion series to compare with the computer-generated fragment ion series of the predicted tryptic peptides.

**Histone Acetyltransferase Assay**—15-μl reactions were carried out in HAT buffer (10 mM Hepes-KOH, pH 7.9, 10 mM MgCl2, 50 mM NaCl, 5 mM dithiothreitol, 10 mM sodium butyrate, 0.25 mg/ml bovine serum albumin, 5% glycerol), using 0.25 μCi of [3H]acetyl-coenzyme A (4.6 Ci/mmol, 250 μCi/ml) per assay and 5 μg of core histones where indicated. Core histones were prepared from HeLa cells as described (18). Histone tail peptides, mimicking the last 25–30 amino acids of the respective tails (5 μg), were also used. HAT reactions were incubated for 45 min at 30 °C. Reactions were analyzed by scintillation counting or fluorography. For visualization by autoradiography, the reactions were terminated by the addition of SDS-sample buffer followed by SDSPAGE using 16.5% Tris-HCl peptide gels (Bio-Rad). After staining of the histones by Coomassie Brilliant Blue, the gels were soaked in Amplify solution (Amersham Biosciences), dried, and subjected to fluorography for 2–14 days.

**Expression of Human Elp3 in Insect Cells**—For expression in insect cells, the hELP3 coding region was cloned into the I-ANNR retroviral vector (22) and transfected into Sf9 cells (Invitrogen). Details of the clone are available on request. HATPase-depleted similar proportions of total IKAP and hELP3 protein (Fig. 1C prior to sample application). Elongator interacted with both RNAPII-A and RNAPII-D.

**Immunostaining**—To determine the subnuclear localization of IKAP and hELP3, we used antibodies, and G11 early elongation complexes were generated as described (16) until after the ultracentrifugation step. The soluble cell extract was then dialyzed into buffer B-50 (50 mM KCl), loaded onto heparin-Sepharose (Amersham Biosciences, Inc.), and eluted stepwise with buffer B-150, B-300, B-450, and B-700. Proteins from the B-300 eluate were pooled, dialyzed into buffer B-50, loaded onto MonoS HR 10/10 column (Amersham Biosciences), and eluted with a linear gradient from 70 to 800 mM salt in buffer B-150, and 1-ml fractions were collected. The Elongator-containing fractions were diluted, applied to Progel-TSK DEAE-5PW (Supelco) equilibrated in buffer B-50, and eluted with a linear gradient from 70 to 800 mM salt in buffer B over 10 CV. Elongator fractions were loaded onto HTP hydroxypatite (Bio-Rad), and the resin was resolved as described above. The purity of the fractions from hydroxypatite was determined by silver staining.

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**Human Elongator Complex**

**Fig. 1. Purification of human core-Elongator.** A. IKAP and human ELP3 (hELP3) proteins co-purify during chromatography. Fractions eluting from DEAE-Sepharose were fractionated by SDS-PAGE, blotted, and probed with antibodies directed against the proteins indicated on the right. Lane 1 contains recombinant human Elp3 from insect cells. B. Similar proportions of IKAP and hELP3 protein are immunorecipitated by anti-IKAP antibody. The input and unbound fractions of protein incubated with protein A-antibody anti-IKAP antibody were fractionated by SDS-PAGE together with 5% of the protein obtained by subsequent peptide elution of the beads. Separated proteins were blotted and probed with antibodies directed against the proteins indicated on the right. C. Purification procedure (left panel) and the most purified core-Elongator fraction (right panel) from the final hydroxyapatite column. Migration of size markers is indicated on the left, and the proteins are designated on the right. The asterisk denotes a protein that was not consistently found in highly purified Elongator fractions and might be a contaminant or loosely associating protein. D. HAT activity and the hELP4 protein do not co-elute with the peak of core-Elongator during MonoQ chromatography. Fractions from MonoQ were resolved by SDS-PAGE, blotted, and probed with antibodies directed against the proteins indicated on the left (upper two panels) or assayed for HAT activity (lower panel).

**Fig. 2. Interaction of Elongator with RNA polymerase II.** A. Early elongation complexes contain Elongator. Proteins isolated with early elongation complexes (EECs) were fractionated by SDS-PAGE, blotted, and probed with antibodies directed against the proteins indicated on the left. The sarcosyl supernatant of G11 EECs is shown in lane 1. Lanes 2 and 3 show different amounts of RNAPII-A as reference. Lane 4 shows proteins remaining in EECs after the stringent sarcosyl wash, and lane 5 shows the proteins present after the mild washes. Note that RNAPII present in EECs as expected is hyperphosphorylated. B. Highly purified Elongator can associate with sarcosyl-washed EECs. Sarcosyl-washed, bead-bound EECs (lane 1) or the control beads containing DNA only (lane 4) were incubated with or without core Elongator as indicated, and EEC-associated proteins were brought down by centrifugation in a pellet (P), separated from the non-associated proteins of the supernatant (S). Proteins were resolved by SDS-PAGE, blotted, and probed with antibodies directed against the proteins indicated on the left. C. Direct association of human Elongator with RNAPII in solution. Elongator and RNAPII were filtrated (as indicated on the left) through Superose 6, either as individual factors (upper panels) or after their mixing (lower panels). The eluted fractions were blotted and probed with antibodies directed against the proteins indicated on the right.

Elongator complexes, while Elongator was quantitatively shifted to elute in later fractions by the association. Collectively, these results indicate that the Elongator HAT complex has the intrinsic ability to associate directly with RNAPII in elongation complexes, suggesting a role for the complex during transcription in human cells.

**Cellular Localization of Elongator Complex**—Elongator localization was determined by immunostaining (Fig. 3). As expected, both IKAP and hELP3 were found predominantly in the cell nucleus. Unexpectedly, however, significant fractions of the proteins localized to the nucleoli and both IKAP and hELP3 were found predominantly in the cytoplasm. By guest on November 13, 2017 http://www.jbc.org/ Downloaded from
suggest that the human Elongator complex is unstable and that the hELP4-containing fractions represent an active form of human Elongator complex remaining after several columns of purification. Such a chromatographic behavior would be strikingly similar to that of yeast Elongator, which can dissociate into two three-subunit subcomplexes during purification (7). These findings prompted us to design an alternative procedure to purify the intact complex by taking advantage of an anti-IKAP immunoaffinity step (Fig. 4).

When the purification procedure that took advantage of affinity chromatography was employed, the subunit composition of human Elongator complex differed significantly from the core complex purified by conventional means (Fig. 4A). We designate this complex human holo-Elongator. The virtually homogenous, immunopurified complex was compared with core Elongator complex in HAT assays. Holo-Elongator had robust HAT activity directed against histone H3 and H4, whereas similar quantities of core Elongator had no activity (Fig. 4B), indicating that the additional subunits confer HAT activity to the holo-Elongator complex. As expected, the activity of holo-Elongator was directed against the tails of histones H3 and H4 (Fig. 4C). These results indicate that the three small holo-Elongator subunits, hELP4, p38, and p30, are required to activate the HAT activity of hELP3 or that one of these proteins has intrinsic HAT activity.

Identification of Elongator Subunits—Four of the subunits of holo-Elongator were identified by a combination of peptide mass fingerprinting and mass spectrometric sequencing. In this way, the presence of IKAP, hELP3, and hELP4 in human holo-Elongator was confirmed. Moreover, the 95-kDa protein, which was also observed in core Elongator (p95; Fig. 1C), was identified as hypothetical protein BAB14193 (NCBI g10434263), a conserved WD40 repeat protein that is the human homologue of yeast Elp2 and mouse StIP1 (10) (Fig. 5). Interestingly, data base searching for protein motifs in hELP2 revealed the presence of a motif (signature 2 motif) found repeated several times in the regulator of chromosome condensation (RCC1) protein. RCC1 has recently been shown to bind to chromatin via association with histones (24), but whether this requires the signature 2 motif is not known.

We conclude that the components of the Elongator complex are well conserved from yeast to humans.

DISCUSSION

In this report, we describe the purification and characterization of human Elongator. We find that human Elongator is remarkably similar to the yeast complex on several levels. First, it consists of homologues of the yeast Elp proteins. We have so far identified human homologues of yeast Elp1, Elp2, Elp3, and Elp4 in the holocomplex, which, like the yeast counterpart, appears to consist of six subunits. The p38 and p30 subunit of the human complex might be encoded by homologues of yeast Elp5 and Elp6. Second, as in yeast, human Elongator complex appears to exist as a core, three-subunit complex (hELP1/IKAP, hELP2, and hELP3) or as a larger, six-subunit holo-Elongator complex. In all likelihood, the human holo-Elongator complex dissociates during purification, at least partly as a consequence of treatment with high salt and MonoQ chromatography, as has previously been demonstrated for the yeast complex (7). Third, human holo-Elongator complex has histone H3 and H4 HAT activity, which has also been observed for the yeast holocomplex. Finally, human Elongator complex has the ability to associate with RNAPII both in solution and in elon-

2 G. S. Winkler, and J. Q. Svejstrup, unpublished results.
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The largest subunit of human elongator is encoded by the IKAP gene. This protein was originally isolated biochemically as a subunit of a large complex containing IκB kinase and proposed to be a scaffold protein for the assembly of these proteins (9). However, IKAP is the closest human homologue of yeast Elp1 with regions of high similarity spread over the entire coding region (4, 9), and recent data seriously question the proposed role of IKAP in IκB kinase signaling (15). Thus, IKAP exists in a complex of the size expected for Elongator and immunoprecipitation of the protein from crude extracts yields a 5–7-subunit complex (15) of a composition similar to the holo-Elongator complex presented here. Importantly, this immunoprecipitated IKAP complex does not include IκB kinase, and the IκB kinase complex still forms and is activated normally in cells where IKAP mRNA and protein levels are reduced to very low levels by antisense oligonucleotides (15). Finally, overexpression of IKAP blocks not only induction of a NF-κB-dependent

Fig. 5. Human ELP2 protein. Shown is the predicted amino acid sequence of the protein encoded by hELP2 and alignment with homologues from other species using ClustalX (26). H.s., Homo sapiens; M.m., Mus musculus; A.t., Arabidopsis thaliana; S.p., Schizosaccharomyces pombe; S.c., Saccharomyces cerevisiae. The mouse Elp2 protein is identical with Stp1 (10). Conserved residues are shaded in light gray, and identical residues are shaded in dark gray.

The indicated region of homology to RCC1 signature 2 was found using PredictProtein (available on the World Wide Web at maple.bioc.columbia.edu/predictprotein/). WD40 repeats are numbered and underlined.
ent reporter gene but also transcription from several NF-κB-independent promoters (15). Taken together, the data thus suggest that IKAP is not involved in IkB kinase signaling but rather plays a role in RNAPII transcription as a component of Elongator.

A splicing site mutation in the gene encoding IKAP causes the severe human recessive disorder, familial dysautonomia (11, 12). This mutation leads to the tissue-dependent expression of a truncated version of the IKAP protein, which results in the poor development, survival, and progressive degeneration of the sensory and autonomic nervous system. The disorder is invariably fatal, with only 50% of patients reaching age 30 years (see Ref. 12 and references therein). The identification of IKAP as a component of the human Elongator complex opens the possibility that the disease might be caused by reduced tissue-specific expression of genes and that it thus could be a transcription disorder. A further intriguing connection between Elongator and human disease stems from the recent finding that amino acid substitutions in the IKAP gene product are not conserved in lower eukaryotes, while StIP1/Elp2 clearly are. Possible explanations to this conundrum include that the disease might be caused by reduced expression of IKAP as a component of the human Elongator complex opens the possibility that the disease might be caused by reduced tissue-specific expression of genes and that it thus could be a transcription disorder. A further intriguing connection between Elongator and human disease stems from the recent finding that amino acid substitutions in the IKAP gene product can significantly increase the risk for bronchial asthma in children (25). Taken together, these connections suggest that cell lines with perturbed Elongator function might be used in genomics approaches to identify genes whose altered expression are causing these diseases.

Intriguingly, the hELP2 subunit of human Elongator is encoded by the homologue of mouse StIP1, which was recently isolated in a two-hybrid screen for proteins that interact with isolated RNAPII and the Imperial Cancer Research Fund service facilities, specifically cell production services, for help. We thank Danny Reinberg for communicating results prior to publication, and we thank Peter Verrijzer and members of the Svejstrup laboratory for comments on the manuscript.

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Purification and Characterization of the Human Elongator Complex
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