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A Multiprotein Complex That Interacts with RNA Polymerase II Elongator*

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A three-subunit Hap complex that interacts with the RNA polymerase II Elongator was isolated from yeast. Deletions of genes for two Hap subunits, *HAP1* and *HAP3*, confer pGKL killer-insensitive and weak Elongator phenotypes. Preferential interaction of the Hap complex with free rather than RNA polymerase II-associated Elongator suggests a role in the regulation of Elongator activity.

Transcription by RNA polymerase II in eukaryotic cells is regulated at all levels, including reorganization of the chromatin template and transcription initiation, elongation, and termination (1). Chromatin reorganization is affected, in part, by histone modifications, such as acetylation (2), deacetylation (3), phosphorylation (4), and methylation (5). Histone acetylation has long been correlated with transcriptional activity (6), and most known histone acetyltransferases (HATs)[1] are believed to act prior to the initiation of transcription. Recently, however, complexes containing HAT activities have been found associated with elongating forms of RNA polymerase II (7, 8). The most extensively characterized of these complexes, termed Elongator, was purified from the yeast *Saccharomyces cerevisiae* and contains three subunits, products of the *ELP1*/*IKI3*, *ELP2*, and *ELP3* genes (8–10). Elp3 is responsible for the HAT activity of the complex and is highly conserved between yeast and man (8). The largest subunit, Elp1, was previously isolated from a genetic screen for resistance to *Kluyveromyces lactis* toxin (11).

Elongator has been found associated only with RNA polymerase II bearing a hyperphosphorylated carboxyl-terminal domain (CTD). It appears to replace the Mediator complex, which interacts with RNA polymerase in the unphosphorylated state during the initiation of transcription. Elongator- and Mediator-polymerase interactions may therefore be reciprocally controlled by the action of CTD kinases (9, 12). Here we describe the isolation and characterization of a three-subunit Hap complex that interacts with Elongator. As the Hap complex is found associated only with free Elongator, it may serve to regulate Elongator-polymerase interaction as well.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—Protein complexes were purified from *S. cerevisiae* strain BJ926 (*α/a trp1/+prc1–126/prc1–126 pep4–3/pep4–3 prb1–1122/prb1–1122 can1/can1*; from Dr. E. Jones, Carnegie-Mellon University, Pittsburgh, PA) grown in YPD (1% yeast extract/2% Bactopeptone/2% glucose (w/v)) (13). *HAP* deletion strains were obtained from Saccharomyces Genome Deletion Project (Research Genetics, Huntsville, AL). Both Δ*hap1* (record number 2150) and Δ*hap3* (record number 6452) deletions are in *S. cerevisiae* strain Hansen BY4741 (*mat a his3D1 leu2D0 met15D0 ura3D0*).

Protein Purification and Sequencing—Purification of Elongator and Hap protein complexes was as described (14). Protein subunits were separated in an SDS-9% polyacrylamide gel, transferred to a polyvinylidiene difluoride membrane (Bio-Rad) in 25 mM Tris base/192 mM glycine/20% methanol/0.01% SDS and stained with Ponceau S. Protein bands were excised and digested *in situ* with trypsin. Peptides were identified by MALDI-reTOF mass spectrometry and by sequencing (15–17).

Anti-Hap2 Antibody Production and Immunoprecipitation—HAP2 was cloned in pET11d between NcoI and *Bam*HI sites and expressed in BL21 (DE3 plysS). Cells were grown to an A_{600} of 0.8 and induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h. Hap2 protein was purified from the insoluble fraction of a cell lysate as described (18) and was used to raise antibodies in rabbits. For immunoprecipitation, crude anti-Hap2 antiserum (250 μl) was coupled to protein A-Sepharose beads (25 μl) (19), incubated for 4 h at 4 °C with MonoQ column fractions (30 μl, sedimented for 5 min at 13,000 rpm in a microcentrifuge before use), washed three times with 100 μl of 20 mM Tris-HCl, pH 7.7/10% glycerol/0.1 mM EDTA/0.2% Nonidet P-40/0.1 mM dithiothreitol/500 mM potassium acetate, and eluted twice with 27.5 μl of 5 M urea for 10 min at room temperature. To the combined eluates was added 35 μl of 5× SDS gel-loading buffer, and one-third of the mixture was applied to a lane of an SDS gel.

pGEL Killer Assay—Killer sensitivity was assayed as described (20) with the following modifications. Parental and deletion strains were grown in YPD overnight, with and without 200 μg/ml of G418. Cells were diluted 10-fold with YPD medium, and 5 μl of the diluted cell suspension was spotted on a YPD plate. *K. lactis* IFO 1267 harboring killer plasmids, pGKL1 and pGKL2, was inoculated on the edge of the cell spot. The plate was incubated at 30 °C overnight to reveal the effect of the secreted killer toxin.

RESULTS AND DISCUSSION

A Novel Protein Complex Associated with RNA Polymerase II Elongator—Elongator was isolated as a complex with RNA polymerase II from the insoluble fraction of a yeast cell extract (9). We have also noted its presence, apparently associated with RNA polymerase II, in the soluble fraction (Fig. 1*A*). Three proteins that comigrated on a MonoQ column with RNA polymerase II from this fraction (marked by dots to the left of the bands in Fig. 1*B*, *fractions 54–60*) were identified by peptide mass fingerprinting and sequencing as the Elongator subunits Elp1, Elp2, and Elp3.

All three Elongator subunits were also found in a second peak from the column that contained very little RNA polymer-

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[1] The abbreviations used are: HAT, histone acetyltransferase; CTD, carboxyl-terminal domain.

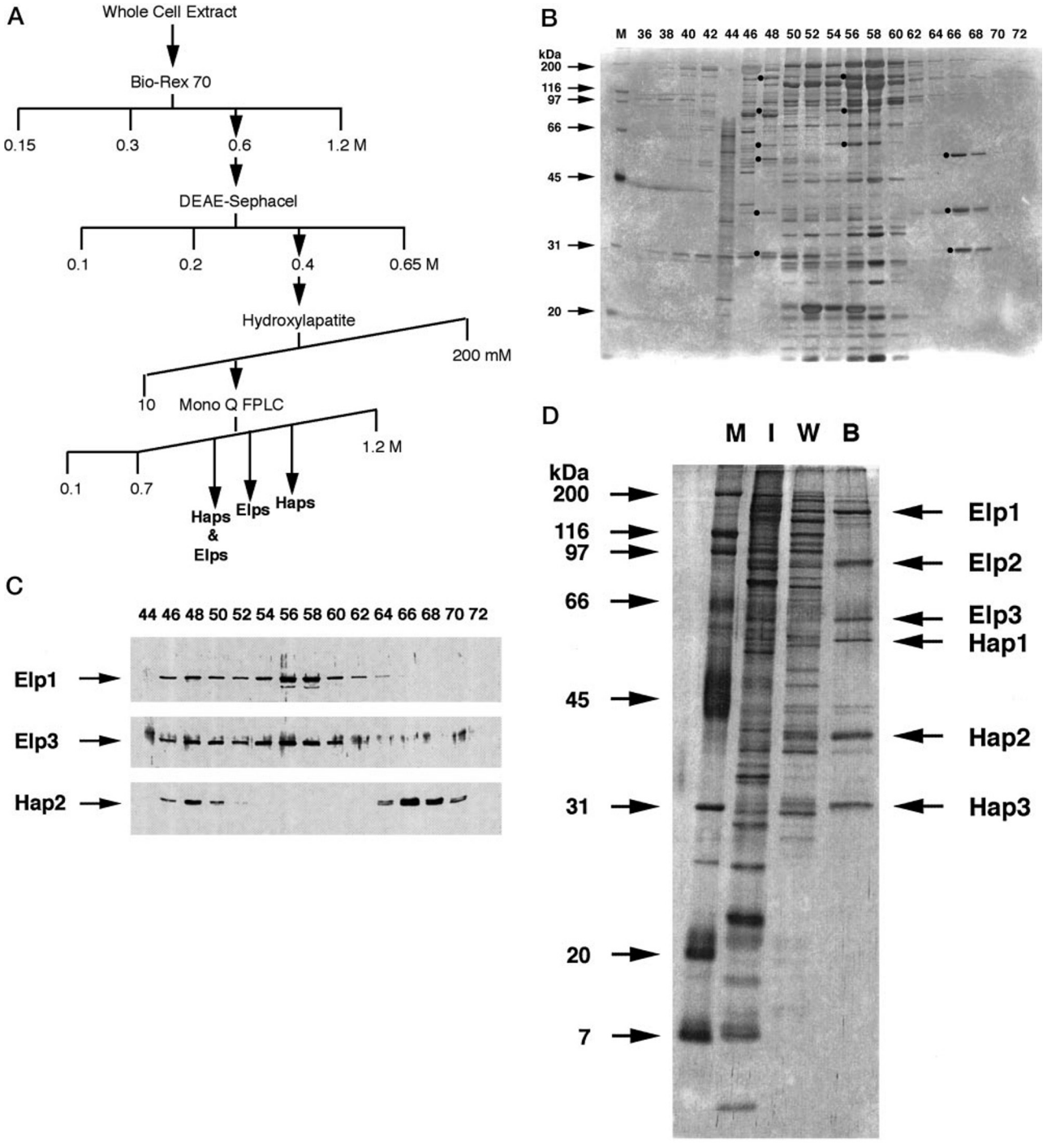

FIG. 1. **Isolation of Hap complexes.** *A*, purification scheme for Hap and Elongator (*Elp*) complexes. *FPLC*, fast protein liquid chromatography. *B*, SDS-polyacrylamide gel electrophoresis of MonoQ fractions. Proteins were revealed by silver staining. Hap and Elp protein bands are marked by *dots* on the *left*. *Fraction numbers* are indicated at the *top*. *M*, molecular mass markers. *C*, immunoblot analysis of MonoQ fractions with anti-Elp1, anti-Elp3, and anti-Hap2 antibodies. *Fraction numbers* are indicated at the *top*. *D*, immunoprecipitation performed on MonoQ fraction 48 with anti-Hap2 antibodies. *M*, molecular mass markers; *I*, input; *W*, wash; *B*, bound and eluted fraction.

ase II (Fig. 1*B*, *fraction 48*). The identity of the subunits in the two peaks was confirmed by immunoblotting with anti-Elp1 and anti-Elp3 antibodies (Fig. 1*C*). Additional proteins, with apparent molecular masses of 51, 38, and 31 kDa, comigrated with Elongator in the second peak (marked along with Elongator subunits by *six dots* to the *left* of the *bands* in Fig. 1*B*, *fraction 48*). We refer to these additional proteins as Hap1, Hap2, and Hap3 (HAT-associated protein).

Identification of Genes for Hap Proteins—Peptide mass fingerprinting by MALDI-reTOF mass spectrometry and peptide sequencing identified Hap1, Hap2, and Hap3 as the products of yeast open reading frames YPL101W, YHR187W, and YMR312W, respectively. YHR187W was previously recovered from a genetic screen for resistance to killing by *K. lactis* toxin (11) and named *IKI1*. No known functional motifs were found in the Hap protein sequences, but homologs were identified in other organisms by data base searches (Fig. 2). Hap1 homologs were particularly widespread, ranging from *Schizosaccharomyces pombe* to *Arabidopsis thaliana*, Drosophila, mice, and humans (Fig. 2*A*).

A Discrete Hap Complex—Three proteins with the same apparent molecular masses as Hap1, Hap2, and Hap3 were pres-

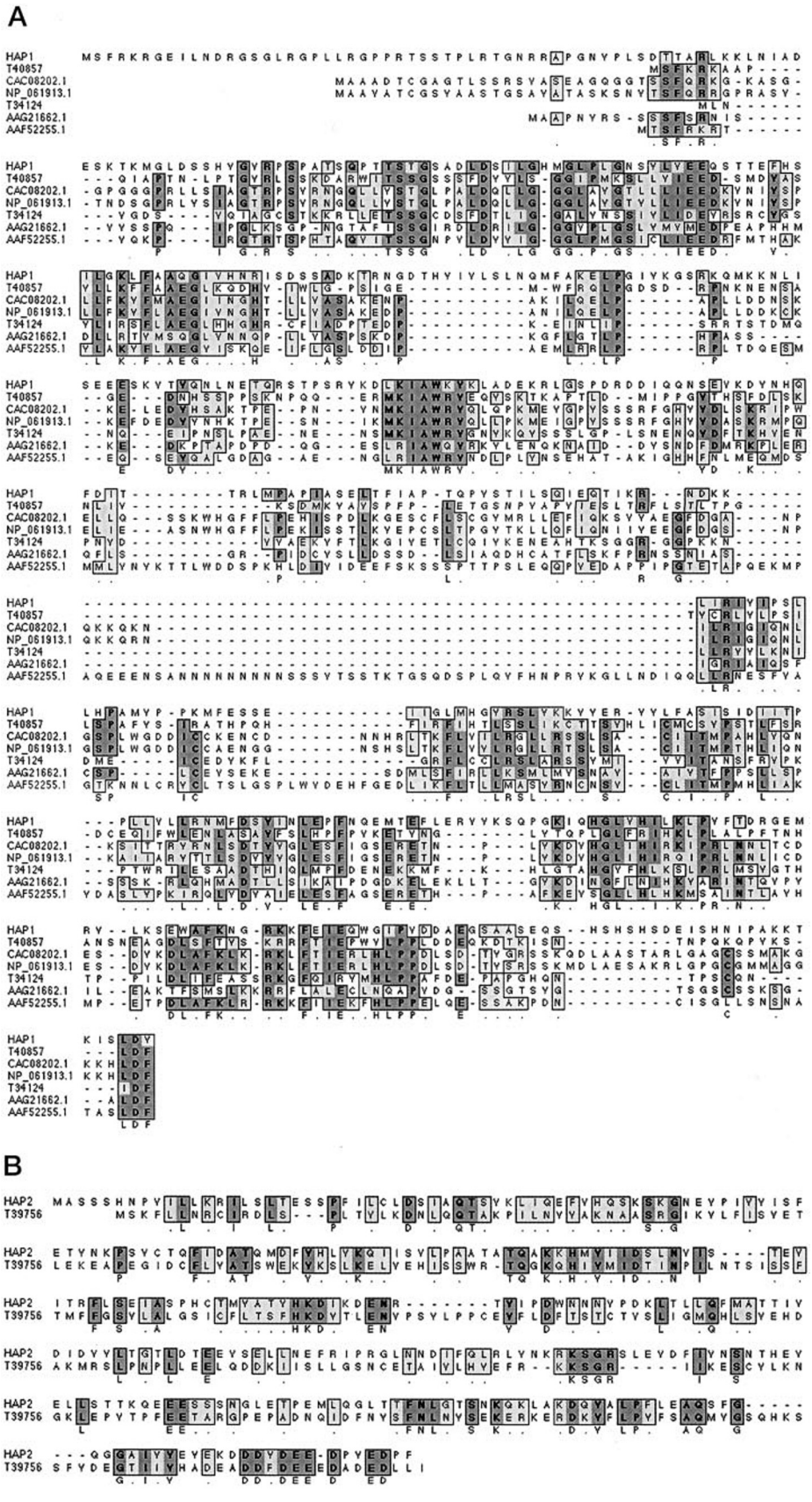

FIG. 2. **Sequences of Haps and homologs.** Sequences were aligned using ClustalW. Accession numbers are shown to the *left* of the sequences. *A*, Hap1 homologs. *T40857*, hypothetical protein SPCC11E10.06c from fission yeast *S. pombe*; *CAC08202.1*, Paxneb protein from mouse; *NP 061913.1*, hypothetical protein from human; *T34124*, hypothetical protein C26B2.6 from *Caenorhabditis elegans*; *AAG21662.1*, hypothetical protein 53156–50996 from *A. thaliana*; *AAF52255.1*, CG6907 gene product from *Drosophila melanogaster*. *B*, Hap2 homologs. *T39756*, hypothetical protein SPBC18E5.05c from fission yeast *S. pombe*.

ent in a third peak from the MonoQ column (Fig. 1*B*, *fraction 66*). This peak was apparently devoid of both Elongator and RNA polymerase II. Identity of the proteins with the three Haps was confirmed by peptide sequencing and was supported by immunoblotting with anti-Hap2/Iki1 antibodies (Fig. 1*C*). Immunoblotting also confirmed the presence of Hap2 in the peak with Elongator (Fig. 1*C*, *fraction 48*). Finally, the interaction of Haps with one another and with Elongator in this peak was supported by coimmunoprecipitation with anti-Hap2 antibodies (Fig. 1*D*).

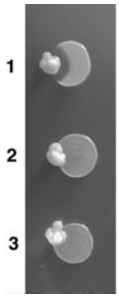

FIG. 3. **Killer-insensitive phenotypes of Δ*hap* yeast strains.** *1*, parental strain; *2*, Δ*hap1*; *3*, Δ*hap3*.

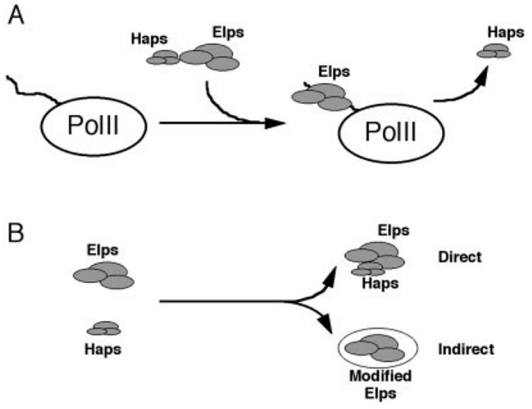

FIG. 4. **The Hap complex may regulate Elongator (*Elps*) function either directly (*A*) or indirectly (*B*).** For simplicity, only RNA polymerase II (*PolII*), Haps, and Elps are indicated.

The Haps can therefore be isolated in two forms, as a six-subunit complex with Elongator and as a complex of the three proteins on their own. Elongator can be recovered in three forms, as a complex with RNA polymerase II, as a complex with Haps, and as a three-subunit complex alone (9). If the interactions of Elongator with Haps and with RNA polymerase II are mutually exclusive, then the Haps may regulate the Elongator-polymerase interaction.

Phenotypes of Δhap1 and Δhap3 Cells—The function of Haps *in vivo* was investigated by deletion of *HAP1* and *HAP3* genes. In view of the identity of *HAP2* with *IKI1* and of the interaction of the Hap complex with Elongator, Iki$^-$ (sensitivity to *K. lactis* toxin (11)) and Elp$^-$ phenotypes were assessed. Both Δ*hap1* and Δ*hap3* strains (labeled *2* and *3* in Fig. 3) showed killer-insensitive phenotypes, whereas the parental strain (labeled *1*) was killer-sensitive. Previously described Elp$^-$ phenotypes, slow start and 6-azauracil sensitivity, were weak but apparent (data not shown). Similar weak Elp$^-$ phenotypes are characteristic of single Δ*elp* mutants and only become more pronounced in the presence of additional mutations (8–10, 21).

We can only speculate as to the role of the Hap complex in transcription. It might keep the HAT activity of free Elongator in check, allowing histone acetylation only in the presence of a transcribing polymerase (Fig. 4*A*). Alternatively, interaction with Haps might render Elongator susceptible to modifications affecting its activity (Fig. 4*B*). These and other possibilities will be addressed in future studies.

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