A novel yeast gene, *ELP2*, is shown to encode the 90-kDa subunit of the Elongator complex and elongating RNA polymerase II holoenzyme. *ELP2* encodes a protein with eight WD40 repeats, and cells lacking the gene display typical *elp* phenotypes, such as temperature and salt sensitivity. Generally, different combinations of double and triple *ELP* gene deletions cause the same phenotypes as single *ELP1*, *ELP2*, or *ELP3* deletion, providing genetic evidence that the *ELP* gene products work together in a complex.

The Elongator complex is a major component of native RNA polymerase II (RNAPII) elongation complexes and co-purifies with elongating RNAPII holoenzyme after its extraction from ternary DNA/RNA/RNAPII complexes (1). Elongator interacts directly with RNAPII and stable association is dependent on the C-terminal domain of the largest polymerase subunit being in a hyperphosphorylated state. Another RNAPII-associated complex, Mediator (2, 3), is exclusively associated with non-phosphorylated RNAPII and is recycled at transcriptional initiation (4). It is therefore an attractive model that Elongator displaces Mediator at the initiation/elongation transition in response to transcription factor IIIH-mediated C-terminal domain phosphorylation and thereafter assists RNAPII during elongation (1). Elongator comprises three subunits of 150, 90, and 60 kDa. The gene encoding p150, *ELP1/KI3*, appears to have no significant homology to protein domains of known function (1). It does, however, have a well conserved *Schizosaccharomyces pombe* homologue of unknown function and, furthermore, might be the yeast counterpart of human IKAP protein (5). The gene encoding p90, *ELP2*, is highly conserved, with likely functional homologues found in many higher eukaryotes, such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and man (6). ELP3 is a histone acetyltransferase, pointing to a role for Elongator in chromatin disruption during transcript elongation. Deletion of *ELP1* or *ELP3* confers a peculiar slow start phenotype, manifested as a pronounced delay in adaptation to new growth conditions. Elongator mutants are also salt- and temperature-sensitive. We have found that these phenotypes are in all likelihood a result of delays in the activation of genes required for growing under the new conditions. For example, activation of the genes *GAL1–10*, *PHO5*, and *ENA1*, which are normally repressed and turned on 50–1000-fold by appropriate growth conditions, is delayed and reduced in the mutants (1, 6).

Here we show that the 90-kDa subunit of Elongator is encoded by the *ELP2* gene, which encodes a protein with eight WD40 repeats. *ELP2* deletion, and, indeed, all combinations of *ELP* deletions, confer phenotypes similar to those previously described for *ELP1* and *ELP3*.

### EXPERIMENTAL PROCEDURES

#### Expression and Purification of Recombinant Elp2

The open reading frame encoding Elp2 was amplified by the polymerase chain reaction from yeast genomic DNA, using primers that introduced a hexahistidine tag and a unique BamHI site at the end encoding the N terminus, and a unique SacII site in the end encoding the C terminus. The sequence of the primers were 5'-GGCCCGGATCCCTTATGCGCACCACATC ACCACACGTGAATGATCATCCTCGGAARGC-3' and 5'-CGGGG TGTGACATTTGATGCTAATGAG TATATACG-3', respectively. Because the full-length protein did not express well in bacteria, a 450-base pair BamHI-EcoRI fragment from the above plasmid (corresponding to codons 1-142) was cloned into the bacterial expression plasmid pGEX-3X (Amersham Pharmacia Biotech), resulting in the fusion of the glutathione S-transferase with a piece of his-tagged open reading frame of *ELP2*. Recombinant glutathione S-transferase-hisElp2 protein fragment was expressed in bacterial DE3 cells by growth at 37 °C to an *A*$_{600}$ of 0.5 and then addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.5 mM. Cells were grown for 3 h after induction. Recombinant Elp2 was purified from the soluble fraction by nickel chromatography (Qiagen).

#### Preparation of Elp2 Antiserum

Purified, recombinant Elp2 protein fragment was fractionated by SDS-polyacrylamide gel electrophoresis and visualized by staining with 0.1% Coomassie Blue R-250. The purified protein was used to immunize rabbits.

#### Distortion of the ELP2 Gene

A cassette encoding the HIS3 or URA3 marker was amplified by the polymerase chain reaction (PCR) from pRS303 or pRS306 (7) using primers that contained 17-mer oligonucleotide sequences for the amplification, flanked by 61-mer sequences that were homologous to those immediately upstream and downstream from the *ELP2* open reading frame, respectively (8). The product from the PCR amplification was used directly to transform diploid wild type W303 (9), or *ELP1*/*elp1* *ELP3*/*elp3 (MATa/a ade2-1/aDE2-1 trp1-1/ trp1-1 can1-100/can1-100 leu2-3, -3, 112/leu2-3, -3, 112 his3-11, 115 his3-11, 15 urs3/urs3 ELP1/elp1-15; URA3 ELP3/elp3-15; LEU2). Transformed cells were plated onto synthetic medium lacking the appropriate amino acids. PCR reactions with primers spanning the *ELP2* gene, and with a primer from within the marker gene to one in the flanking sequence of *ELP2*, were performed on several His*+* (or Ura*) transformers. Strains carrying the correct mutation were sporulated, and tetrads were dissected on YPD agar and replica-plated onto synthetic media lacking uracil, leucine, or histidine. Strains are listed in Table 1. Phenotypic assays were performed as described (1, 6).

#### Other Methods

Purification of Elongator and elongating RNAPII holoenzyme, as well as matrix-assisted laser desorption ionization/time of flight mass spectrometry methods used for the identification of Elp2,
have been described previously (1, 6). Immunoblotting was as described (10). The anti-Elp2 antibody was used at a final dilution of 1:250 to 1:500, and the secondary antibody/detection reagent was a goat anti-rabbit alkaline phosphatase conjugate (Bio-Rad), or an anti-rabbit horseradish peroxidase conjugate (Amersham Pharmacia Biotech). Silver staining was performed as described (11).

RESULTS AND DISCUSSION

Peptide mass fingerprinting using matrix-assisted laser desorption ionization/time of flight mass spectrometry (12–14), was used to identify the 90-kDa subunit of the Elongator complex (1) as the product of the previously defined YGR200c ORF on yeast chromosome XII (predicted mass: 89 kDa). Antiserum against the recombinant protein was raised in rabbits and tested for reactivity toward the subunits of essentially homogenous yeast Elongator (from the Mono Q step of purification) by immunoblotting. During chromatography of the Elongator preparation, immune reactivity precisely co-eluted with the other subunits of Elongator, Elp1 and Elp3 (Fig. 1A, and data not shown). Moreover, immune serum, but not pre-immune serum, specifically recognized a protein with the same mobility as the 90-kDa protein evident from silver staining of a RNA polymerase II holoenzyme preparation (Fig. 1B). We conclude that the open reading frame, which we term the ELP2 gene for Elongator Protein 2, encodes the 90-kDa subunit of Elongator and elongating RNAPII holoenzyme.

Data base searching revealed that the ELP2 gene product has eight domains with homology to WD40 repeats (Fig. 2A). These repeats are found in all eukaryotes and are implicated in a variety of crucial functions, likely by enabling specific protein–protein interactions (15). Importantly, the homology of the Arabidopsis and S. pombe proteins to Elp2 was not confined to the WD40 repeats, indicating that these proteins might indeed be functional homologues (Fig. 2B). Interestingly, a number of protein complexes involved in chromatin remodeling, such as CAP-1 (16), NURF (17), histone deacetylase complexes (18), and the B-type HAT complex in yeast (19), all contain proteins with WD40 repeats. The precise function of the WD40 domains in these complexes remains to be established, but it is an obvious possibility that they might enable a proper alignment of the respective enzymatic subunit they support with histone tails (20, 21).

To test the requirement for Elp2 in yeast, a diploid ELP1/elp1 ELP3/elp3 strain was made in which one copy of the entire ELP2 gene was replaced with HIS3. This approach made it possible to easily compare the consequences of ELP2 deletion with deletion of the other ELP genes, and to get an impression of the consequence of double and triple ELP deletion. The resulting ELP1/elp1 ELP2/elp2 ELP3/elp3 diploid strain was induced to sporulate, and the resulting tetrads were dissected. The same slow start phenotype previously described for elp1Δ, elp3Δ, and the elp1Δ elp3Δ double mutant (1, 6), was seen for elp2Δ, and also for the other combinations of ELP deletions, including the triple delete strain (Fig. 3, and data not shown). With all the different combinations of ELP delete strains available, we then did a number of phenotypic assays in order to investigate differences and similarities among the constructed strains. As shown previously for elp1Δ and elp3Δ (1, 6), strains harboring an ELP2 deletion, including double and triple mutants with other ELP deletions, were unable to survive at 39 °C (Fig. 4).

We then took advantage of growth conditions under which elp1Δ and elp3Δ deletion had previously been found to confer mild growth disadvantages, with the expectation that possible differences in the degree of severity of the different mutations might be detected in this way. elp1Δ strains fail to grow on 1 M NaCl (YPD) plates when plated from midlog cultures, in all likelihood due to delayed and reduced transcription of the gene encoding the sodium pump, ENA1 (1). All the other elpΔ strains also failed to grow under these conditions when plated from midlog cultures (data not shown). By contrast, we observed that growth of elpΔ strains on high salt plates was only slightly affected when cells were plated from cultures grown into stationary phase rather than midlog phase, presumably because ENA1 together with other stress genes becomes expressed as cells enter stationary phase growth (23). We grew cells to stationary phase and plated on 1 M NaCl plates in the hope that a graduation of severity of elp mutations could be detected, if it existed. However, as can be seen in Fig. 5A, all elp strains behaved similarly in this phenotypic assay. They all grew slightly less well than wild type. We next took advantage of a slow growth adaptation phenotype observed for elp strains on

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**TABLE I**

| Strain     | Genotype                      | Reference  |
|------------|-------------------------------|------------|
| W303–1a    | MATa, ura3, leu2–3, -112, his3–11, -15, trp1–1, ade2–1 can1–100 | (9)        |
| JSY103     | As W303–1a, but elp1Δ::URA3   | (1)        |
| JSY334     | As W303–1a, but elp2Δ::HIS3   | This study |
| JSY304     | As W303–1a, but elp2Δ::URA3   | This study |
| JSY130     | As W303–1a, but elp3Δ::URA3   | (6)        |
| JSY160     | As W303–1a, but elp3Δ::LEU2   | (6)        |
| JSY103     | As W303–1a, but elp1Δ::URA3, elp3Δ::LEU2 | This study |
| JSY161     | As W303–1a, but elp2Δ::HIS3, elp3Δ::LEU2 | This study |
| JSY162     | As W303–1a, but elp1Δ::URA3, elp2Δ::HIS3, elp3Δ::LEU2 | This study |

**FIG. 1.** Anti-Elp2 antibodies specifically cross-react with the 90-kDa subunit of Elongator and elongating RNAPII holoenzyme. A, purified Elongator (2 μl of indicated Mono Q fractions; Ref. 1) was analyzed in a 10% SDS-polyacrylamide gel and visualized by staining with silver (the subunits of Elongator are indicated at the left), or transferred to nitrocellulose and detected with anti-Elp1, -Elp2, or Elp3 antibody as indicated. B, purified RNA polymerase II holoenzyme is shown on the left (S, silver stain) with the subunits of RNAPII and Elp proteins indicated. A Western blot of holoenzyme probed with pre-immune serum (PI) and anti-Elp2 antibodies (Elp2 ab) is shown on the right.
Fig. 2. Elp2 homologies. A, alignment of the 8 WD40 domains found in Elp2. Shaded boxes above sequences denotes non-conserved spacer residues, denoted by asterisks. The precise alignment was done by eye according to the rules described by Neer et al. (24). Italized letters denote residues that fit the consensus. B, alignment of the predicted amino acid sequences of Elp2 from Saccharomyces cerevisiae (cerevisiae), S. pombe (pompe), and A. thaliana (Arabidopsis), with the eight WD40 repeats indicated. Residues that are identical (dark grey) and similar (light grey) between the proteins are indicated. Note the homology also outside the WD40 domains. The overall homology between the proteins is 34.7% identity/50.4% similarity (S. cerevisiae/S. pombe), 25.4%/37.3% (S. cerevisiae/A. thaliana), and 27.1%/38.0% (A. thaliana/S. pombe).

Fig. 3. Deletion of ELP2. elp2Δ mutants have a slow start phenotype similar to that of other elp mutants. Examples of tetrads from dissection of elp1Δ/elp1 elp2Δ/elp2 elp3Δ/elp3 diploid strain are shown after 2–3 days of incubation at 30 °C. The indicated genotypes were determined by replica plating onto SD media lacking leucine, histidine, or uracil.

Fig. 4. elp2 mutation confers typical elongator phenotypes. elpΔ strains are temperature-sensitive. Cells of the indicated genotype were streaked on YPD plates and incubated at either 30 °C or 39 °C for 3–5 days.

Fig. 5. All elp strains have similar phenotypes. A, similar severity of elpΔ mutations for growth under high salt conditions. Strains of the indicated genotype were grown to late log phase/stationary phase (overnight) and plated in serial dilution on a YPD plate containing 1 M NaCl. The plate is shown after 2–3 days of incubation at 30 °C. B, similar severity of elpΔ mutations for growth on galactose. Strains of the indicated genotype were grown to midlog phase and plated in serial dilution on YP galactose. The plate is shown after 2–5 days of incubation at 30 °C.

galactose (1, 6). Again, we expected that if differences in severity of the different elp mutations existed, they would be detectable by this assay (Fig. 5B). All elpΔ strains grew slower than...
wild type, but no significant differences in growth rate between elpΔ strains were observed on galactose either.

The following general conclusions can be drawn from these experiments. First, the phenotypes of elp1Δ, elp2Δ, and elp3Δ strains are very similar. Second, the disruption of any two, or all three, ELP genes in the same cell does not confer a dramatic new phenotype that we have so far been able to find. These results clearly demonstrate that the products of the ELP genes work together in a complex in vivo and indicate that the functional integrity of Elongator is compromised by deletion of any one of these genes. We have recently found that more or less the full range of elp phenotypes are conferred by point mutations which severely diminish the HAT activity of Elp3 in vitro. It is therefore tempting to speculate that a major purpose of the elongator complex in vivo is to serve as a vehicle for the HAT activity of Elp3, and that deletion of any one of the ELP genes leads to loss of the structural integrity of Elongator, leading to loss of productive Elongator HAT activity below a certain threshold, which, in turn, results in the growth defects described.

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The Elp2 Subunit of Elongator and Elongating RNA Polymerase II Holoenzyme Is a WD40 Repeat Protein

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