Two Small Subunits in Arabidopsis RNA Polymerase II Are Related to Yeast RPB4 and RPB7 and Interact with One Another*

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An Arabidopsis cDNA (AtRPB15.9) that encoded a protein related to the RPB4 subunit in yeast RNA polymerase II was cloned. The predicted molecular mass of 15.9 kDa for the AtRPB15.9 protein was significantly smaller than 25 kDa for yeast RPB4. In SDS-PAGE, AtRPB15.9 migrated as the seventh or eighth largest subunit (i.e. apparent molecular mass of 14–15 kDa) in Arabidopsis RNA polymerase II, whereas RPB4 migrates as the fourth largest subunit (i.e. apparent molecular mass of 32 kDa) in yeast RNA polymerase II. Unlike yeast RPB4 and RPB7, which dissociate from RNA polymerase II under mildly denaturing conditions, plant subunits related to RPB4 and RPB7 are more stably associated with the enzyme. Recombinant AtRPB15.9 formed stable complexes with AtRPB19.5 (i.e. a subunit related to yeast RPB7) in vitro as did recombinant yeast RPB4 and RPB7 subunits. Stable heterodimers were also formed between AtRPB15.9 and yeast RPB7 and between yeast RPB4 and AtRPB19.5.

The 12 subunits that make up yeast RNA polymerase II have been firmly established (1, 2), but the subunit structures of RNA polymerase II from other eukaryotes have not been completely resolved. Human, Arabidopsis, and fission yeast RNA polymerase II enzymes have been reported to contain subunits related to most of the subunits found in yeast RNA polymerase II (2–9), but one or more subunits found in the yeast enzyme have yet to be identified in other eukaryotic enzymes. One of these elusive subunits is the equivalent of yeast RPB4 (referred to as ScB16, based on the apparent molecular mass in SDS-PAGE), which is found only in RNA polymerase II and is known to dissociate along with RPB7 (referred to as ScB16, based on apparent molecular mass in SDS-PAGE) from yeast RNA polymerase II under mildly denaturing conditions or upon non-denaturing gel electrophoresis (10–12). A subunit related to RPB7 has been identified in both human and Arabidopsis RNA polymerase II (6, 13), but a subunit related to RPB4 has not been reported for human, plant, or fission yeast enzymes (2, 7, 9). Furthermore, in purified human, plant, and fission yeast RNA polymerase II enzymes, there is no subunit like yeast RPB4 that migrates with an apparent molecular mass of 32 kDa (9, 14–18).

Here we report on the sequence of an Arabidopsis cDNA, AtRPB15.9, which encodes a protein related to yeast RPB4. Antibodies raised against the AtRPB15.9 recombinant protein were used to confirm the existence of this subunit in a variety of purified plant RNA polymerase II enzymes. DEAE-Sephadex chromatography was used to determine if the plant subunit dissociates from RNA polymerase II under mildly denaturing conditions. A renaturation protocol with recombinant yeast RPB4 and RPB7 subunits and related subunits from Arabidopsis was used to determine if these subunits formed stable complexes in vitro.

EXPERIMENTAL PROCEDURES

Isolation of AtRPB15.9 cDNA Clones from Arabidopsis—An EST clone from an oilseed rape (Brassica napus) cDNA library (GenBank™ accession number H07557) was identified that encoded a protein related to yeast RPB4 and a human EST clone (GenBank™ accession number W87845). The B. napus EST clone was provided by Dr. Tae-Ju Cho (Department of Biochemistry, Chung-Buk National University, Cheong-Ju, South Korea) and was used to screen 220,000 plaque-forming units of an Arabidopsis thaliana (ecotype Columbia) suspension culture cell cDNA library in ZAP II (19) using standard techniques (20, 21). The cDNA library was provide by Dr. Michele Alexo.2 Twenty-three positive clones were identified and purified. Most of the clones were approximately the same size, and two of these were sequenced. The two clones contained complete ORFs with identical sequences, except that the two clones had 5’- and 3’-untranslated regions of different lengths, and one clone contained an unprocessed intron. The cDNA clone lacking the intron is referred to as AtRPB15.9.

Genomic Southern and Northern Analysis—A. thaliana (ecotype Columbia) genomic DNA was isolated, digested with restriction enzymes, subjected to electrophoresis in 0.8% agarose, and blotted onto a Hybond™-N+ membrane (Amersham Life Science, Inc.) using standard techniques (20, 22). The AtRPB15.9 cDNA was labeled with 32P using the Prime-a-Gene labeling system (Promega Corp., Madison, WI). The labeled probe was hybridized to the Southern blot in 6× saline/sodium phosphate/EDTA (20), 1% nonfat dry milk, 1% SDS, and 0.5 mg/ml herring testes DNA at 68 °C. After hybridization, membranes were washed in 2× SSC (1× SSC is 0.15 M NaCl + 0.015 M sodium citrate) and 0.1% SDS for 15 min at 25 °C, 0.5× SSC and 0.1% SDS for 15 min at 25 °C, and 0.2× SSC and 1.0% SDS for 30 min at 50 °C.

Northern blotting was carried out with 2 μg of poly(A)+ RNA isolated from A. thaliana suspension culture cells (23). Total RNA was isolated using a standard protocol (21), and poly(A)+ RNA was prepared from total RNA using the PolyATtract™ mRNA isolation system I (Promega Corp.). Poly(A)+ RNA was denatured with glyoxal and Me3SO, subjected to electrophoresis in a 2.5% agarose gel (composed of a 2:1 blend of NuSieve GTG-agarose (FMC, Rockland ME) to DNA grade agarose (Fisher Biotech, Fair Lawn NJ)), and blotted onto a Hybond™-N+ membrane using standard protocols (20). Hybridization

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The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; EST, expressed sequence tag; RPB or B, RNA polymerase B; ORF, open reading frame; PCR, polymerase chain reaction; NTA, nitritotriacetic acid.
Schuell) were made with step fractions (80, 140, and 300 mM (NH₄)₂SO₄) steps were dialyzed to 20 mM Tris-HCl, pH 7.9, 1 mM EDTA, 1 mM dithiothreitol, 50 mM (NH₄)₂SO₄, and 10% glycerol and dot-blotted. Dot blots were incubated with affinity-purified His-tagged BnRPB15.9, followed by anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Amersham) and a chemiluminescence reagent (NEN Life Science Products).

1. Comparison of the amino acid sequence of the Arabidopsis RPB15.9 subunit with RPB4 (ScB32) and human (HsRPB4). Amino acids that are identical or similar in at least two species are shaded with black or gray, respectively.

RNA Polymerase II Purification and Treatments—RNA polymerase II enzymes were purified as described previously (8, 18, 28). Purified wheat germ RNA polymerase II (1.5 mg) in 3 ml of storage buffer (28) was dialyzed overnight against 1 liter of mildly denaturing buffer containing 1.2 mM urea at 4 °C (11). The dialyzed enzyme was chromatographed on a 12 × 0.8-cm column of DEAE-Sephadex A25 with a 40-ml linear gradient of 80–350 mM ammonium sulfate in buffer containing 1.2 mM urea (11). Chromatographic fractions (0.7 ml) were dialyzed against 20 mM HEPES, pH 7.9, 0.1 mM EDTA, 0.1 mM diethiothreitol, and 50% (v/v) glycerol, assayed for RNA polymerase activity (18), and analyzed in SDS-PAGE (26).

Renaturation and Analysis of Yeast and Arabidopsis RNA Polymerase II Subunits—ORFs encoding Arabidopsis AtRPB15.9 and AtRPB19.5 and yeast RBP4 (ScB32) and RBP7 (ScB16) were amplified from Arabidopsis cDNA clones or yeast genomic DNA using Pwo DNA polymerase (Boehringer Mannheim). PCR products were ligated into pET-16b (Novagen, Madison WI) between NdeI and BamHI sites of pET-16b (Novagen, Madison WI) between NdeI and BamHI sites for plasmids that encode His-tagged subunits or between NeoI and BamHI for plasmids that encode untagged subunits. All recombinant RNA polymerase subunit purifications were accumulated as insoluble products in E. coli. Recombinant subunits were renatured, and subunit interactions were analyzed on Ni²⁺-NTA agarose columns as described by Larkin and Guilfoyle (29) with the exception that untagged subunits were added at 2-fold molar excess to His-tagged subunits. Renatured AtRPB15.9 and AtRPB19.5 complexes were analyzed by gel filtration chromatography using a 120-ml HiPrep 16/60 Sephacyrl S-100 column (Pharmacia Biotech Inc.). The column was equilibrated in 50 mM Tris-HCl, pH 7.9, 200 mM KCl, 10 mM MgCl₂, 10 mM ZnCl₂, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 10% glycerol (29). The column was run at a flow rate of 0.4 ml/min, and 2.4 ml fractions were collected and analyzed in 10% gels (26). Molecular masses of subunits were calculated as recommended by Stellwagen (30).

RESULTS

The Arabidopsis AtRPB15.9 Subunit—A search of the EST data base revealed B. napus (oilseed rape; GenBank™ accession number H07557) and Brassica rapa (Chinese cabbage; GenBank™ accession number L58192), EST clones that encoded proteins related to the yeast RPB4 or ScB32 RNA polymerase II subunit (31). The B. napus EST clone was used as a probe to isolate cDNA clones from an A. thaliana suspension culture cell cDNA library (19). An Arabidopsis cDNA clone (AtRPB15.9) was sequenced that contained an ORF encoding a protein of 15.9 kDa with a pI of 4.9. The amino acid sequences encoded by the B. napus EST cDNA clone and the Arabidopsis cDNA were 97% identical (data not shown). ESTs were also identified for human cDNA clones (Gen-
enzymes, polyclonal antibodies were raised against the is found in polymerase II from a variety of plants. Fig. 3 antibodies were used to probe blots of purified RNA polymerase recombinant protein (BnRPB15.9), and affinity-purified RNA from Arabidopsis suspension culture cells. Molecular size markers are indicated in kilobases to the left. A 32P-labeled Arabidopsis RPB15.9 cDNA probe was used in both Southern and RNA blots.

BankTM accession numbers U85510 and U89387) related to yeast RPB4. The AtRPB15.9 protein is 18.8 and 37.0% identical to yeast RPB4 (ScB32) and human RPB4, respectively (Fig. 1). In contrast to the Arabidopsis and human proteins, yeast RPB4 contains a highly charged insertion of 70 amino acids within the middle of the protein. This insertion results in a yeast RPB4 subunit that contains 221 amino acids with a molecular mass of 25.4 kDa (31), whereas the Arabidopsis protein contains 139 amino acids with a molecular mass of 15.9 kDa, and the human protein contains 142 amino acids with a molecular mass of 16.3 kDa.

On Southern blots, the AtRPB15.9 cDNA probe hybridized to single bands of Arabidopsis (ecotype Columbia) genomic DNA that was digested with a variety of restriction endonucleases (Fig. 2A). These results suggest that the AtRPB15.9 protein is encoded by a single copy gene in Arabidopsis. On Northern blots with poly(A)+ RNA prepared from Arabidopsis suspension culture cells, the AtRPB15.9 cDNA probe hybridized to a mRNA of about 800 bases (Fig. 2B). The size of this mRNA is appropriate for encoding a protein of 15.9 kDa and is in agreement with size estimates of cDNA inserts in cDNA clones encoding AtRPB15.9.

Identification of the AtRPB15.9 Subunit in Arabidopsis RNA Polymerase II and Related Subunits in Other Plant RNA Polymerase II Enzymes—To determine if the AtRPB15.9 protein is found in Arabidopsis and other plant RNA polymerase II enzymes, polyclonal antibodies were raised against the B. napus recombinant protein (BnRPB15.9), and affinity-purified antibodies were used to probe blots of purified RNA polymerase II from a variety of plants. Fig. 3B (lanes 2–7) shows that affinity purified anti-BnRPB15.9 antibodies bind to a subunit of about 14–15 kDa in Arabidopsis, cauliflower, soybean, pea, tobacco, and wheat RNA polymerase II, which represents the seventh or eighth largest subunit in each enzyme. No subunit in purified plant RNA polymerase I or III reacted with the anti-BnRPB15.9 antibody (data not shown). In wheat RNA polymerase II (Fig. 3A, lane 7), the subunits were more clearly resolved and/or efficiently stained with silver, and the eighth largest subunit in this enzyme bound to the BnRPB15.9 antibodies. The apparent molecular mass of this plant subunit (i.e. 14–15 kDa) in the gel system used here (26) is slightly smaller than that observed with the Laemmli gel system (32), where this subunit migrates as the eighth largest subunit with an apparent molecular mass of 17.0 kDa (15, 18). The recombinant AtRPB15.9 protein migrated with a similar molecular mass of 15.3 kDa in this SDS-PAGE system (Fig. 3, lane 1). With similar amounts of RNA polymerases applied to the SDS gel, the antibody binding signal was approximately equal for each RNA polymerase II analyzed. The signal with the more distantly related monocotyledon wheat, however, was less than that observed with dicotyledon RNA polymerase II enzymes (i.e. Arabidopsis, cauliflower, soybean, pea, and tobacco). Results with purified plant RNA polymerase II indicate the RPB4-related subunit has a mobility about half the size of yeast RPB4 (i.e. 14–17 kDa in plants, depending on the gel system, and 32 kDa in yeast).

The RPB15.9 Subunit Does Not Dissociate from Wheat RNA Polymerase II under Mildly Denaturing Conditions—RPB4 (ScB32) and RPB7 (ScB16) dissociate from yeast RNA polymerase II that has been incubated in 1.2 M urea (11). To determine if the RPB4-like subunit dissociates from wheat RNA polymerase II under mildly denaturing conditions, purified wheat RNA polymerase II was treated with 1.2 M urea and chromatographed on DEAE-Sephadex using a linear salt gradient containing 1.2 M urea as described by Ruet et al. (11). The chromatographic fractions were analyzed by SDS-PAGE and protein blotting with an anti-BnRPB15.9 antibody. Fig. 4 shows that little if any wheat RPB17 subunit (the RPB4-like subunit) dissociates from the enzyme under these conditions as determined by Coomassie Blue staining of subunits (Fig. 4A) and immunoblotting (Fig. 4B). Furthermore, there is no apparent dissociation of the wheat RPB21 subunit (i.e. the wheat subunit related to yeast RPB7) (13) as determined by Coomassie Blue staining under these mildly denaturing conditions.
This contrasts with yeast RPB4 and RPB7, which appear to dissociate as a complex from the enzyme under mildly denaturing conditions and are resolved from an RNA polymerase II core on DEAE resins (11, 12). Recombinant reconstituted Arabidopsis RPB15.9/RPB19.5 complexes were subjected to the same chromatographic procedure on DEAE-Sephadex. The reconstituted subunits failed to bind to the DEAE-Sephadex column under these conditions and were recovered in the breakthrough fractions (80 mM (NH₄)₂SO₄) (data not shown). These results suggest that plant RPB4 and RPB7 subunits are more stably associated with wheat germ RNA polymerase II compared with yeast RPB4 and RPB7.

To further test the stable association of plant RPB4 and RPB7 subunits with the enzyme, wheat and Arabidopsis RNA polymerase II were pretreated with 2 mM urea for 3 h and chromatographed on DEAE-Sephadex columns in the presence of 2 mM urea (12). Recombinant reconstituted Arabidopsis RPB15.9/RPB19.5, reconstituted recombinant and Ni²⁺-NTA purified Arabidopsis His-tagged RPB15.9/RPB19.5, and purified wheat germ and Arabidopsis RNA polymerase II were each pretreated with 2 mM urea and chromatographed on DEAE-Sephadex. Samples were applied at 80 mM (NH₄)₂SO₄ and step-eluted at 140 mM and 300 mM (NH₄)₂SO₄ in buffer containing 2 mM urea (12). The SDS gel in Fig. 5A shows that the bulk of the Arabidopsis reconstituted recombinant RPB4/RPB7 subunits failed to bind to DEAE-Sephadex and eluted in the 80 mM (NH₄)₂SO₄ breakthrough fractions. In contrast, no RPB4 or RPB7 subunit was detected in the breakthrough fractions with wheat or Arabidopsis RNA polymerase II, and the bulk of the enzyme eluted at 300 mM (NH₄)₂SO₄. To confirm that RPB4 and RPB7 subunits were stably associated with wheat and Arabidopsis RNA polymerase II enzymes that were treated with 2 mM urea, dot blots of the peak fractions from the 80, 140, and 300 mM (NH₄)₂SO₄ steps were tested with affinity-purified antibodies raised against BnRPB15.9 and cauliflower RPB19.5 (anti-RPB7). Results in Fig. 5B confirm that RPB4 and RPB7 subunits in the plant enzymes did not dissociate from the enzymes pretreated with 2 mM urea and subjected to chromatography on DEAE Sephadex in the presence of 2 mM urea.

Results from two additional experiments (data not shown) support the above experiments on the stable association of RPB4/RPB7 subunit in wheat RNA polymerase II. First the wheat enzyme was pretreated with 2 mM urea and applied to a phosphocellulose column in buffer (the same buffer that was used for DEAE-Sephadex chromatography) containing 75 mM (NH₄)₂SO₄ and 2 mM urea. The column was washed with that application buffer, and RNA polymerase was step-eluted in buffer containing 150 mM (NH₄)₂SO₄ and 2 mM urea. SDS gel electrophoresis and dot-blotting with anti BnRPB15.9 and anti-cauliflower RPB19.5 antibodies showed that the plant RPB4 and RPB7 subunits eluted exclusively with RNA polymerase at 150 mM (NH₄)₂SO₄. Under the same conditions, Arabidopsis reconstituted recombinant RPB4/RPB7 subunits failed to bind to the phosphocellulose column, and these subunits were recovered in the breakthrough fractions. Second, wheat RNA polymerase II, which was step-eluted from the phosphocellulose column, was reacted with affinity-purified BnRPB15.9 antibodies in 2 mM urea. SDS gel electrophoresis of the immuno-precipitate (recovered on a protein A resin) revealed that all subunits were present and that there was no preferential immunoprecipitation of RPB4/RPB7 subunits.

In Vitro Interactions with Recombinant Arabidopsis AtB15.9 and AtB19.5 and Yeast RPB4 (ScB32) and RPB7 (ScB16) Subunits—Yeast RPB4 and RPB7 are thought to interact with one another and to dissociate and associate with RNA polymerase II as a heterodimer (10–12). To determine if yeast RPB4 and
RPB7 and Arabidopsis AtB15.9 and AtB19.5 subunits formed stable heterodimers in vitro, recombinant subunits were expressed in E. coli, recovered as insoluble proteins, and renatured as described by Tang et al. (33) and Larkin and Guilfoyle (29). After recombinant subunits were solubilized in a denaturing buffer containing guanidine hydrochloride, single subunits and various combinations were renatured by dialysis against buffers lacking guanidine hydrochloride. In these renaturing experiments, yeast RPB4 (ScB32) and AtB15.9 contained a His tag on their amino termini, which facilitated purification of soluble, renatured complexes on Ni²⁺-NTA agarose columns. Yeast RPB7 (ScB16) and AtB19.5 lacked a His tag and were retained on the Ni²⁺-NTA columns only if complexed with a His-tagged ScB32 or His-tagged AtB15.9 subunit.

Based on a number of previous experiments (10–12), it was expected that recombinant yeast RPB4 and RPB7 subunits would interact in vitro. When His-tagged RPB4 (ScB32) was subjected to the renaturation protocol, most of the subunit was recovered in a soluble form and could be purified by Ni²⁺-NTA affinity chromatography (Fig. 6A). In contrast, most of the RPB7 (ScB16) subunit precipitated during the renaturation protocol, and the small amount of soluble protein that was recovered failed to bind to the Ni²⁺-NTA resin (Fig. 6B). When RPB7 was renatured in the presence of His-tagged RPB4, a larger amount of RPB7 remained soluble after dialysis, and this was retained on the Ni²⁺-NTA resin (Fig. 6C). These results indicated that yeast RPB4 and RPB7 interact with one another and form stable complexes in vitro.

When His-tagged AtB15.9 was subjected to the renaturation protocol by itself, the subunit precipitated, and little if any of the subunit was recovered in a soluble form (Fig. 7A). Most of the AtB19.5 subunit was recovered in a soluble form when subjected to the renaturation protocol, but this untagged subunit failed to bind to the Ni²⁺-NTA resin (Fig. 7B). When His-tagged AtB15.9 and AtB19.5 subunits were combined and renatured, both subunits were recovered in a soluble form and bound to the Ni²⁺-NTA resin (Fig. 7C). Thus, Arabidopsis AtB15.9 and AtB19.5 subunits interact with one another and form stable complexes like their RPB4 and RPB7 counterparts in yeast.

Another set of experiments was carried out to determine if Arabidopsis His-tagged AtB15.9 could form stable complexes with yeast RPB7 (ScB16) and if yeast His-tagged RPB4 (ScB32) could form stable complexes with Arabidopsis AtB15.9. When His-tagged AtB15.9 and yeast RPB7 were renatured together, a small fraction of the subunits remained soluble and bound to the Ni²⁺-NTA resin (Fig. 8A). When yeast His-tagged RPB4 and AtB19.5 were combined and renatured, the bulk of the subunits remained soluble and bound to the Ni²⁺-NTA resin (Fig. 8B). These results indicate that RPB4 or RPB4-like and RPB7 or RPB7-like subunits can form stable mixed (i.e. plant/yeast) heterodimers in vitro.
Interactions between RNA Polymerase II Subunits

Experiments, both subunits lacked a His tag, and therefore, the His tag itself cannot be responsible for the interactions observed between the *Arabidopsis* RPB4-like and RPB7-like subunits.

**DISCUSSION**

Yeast RNA polymerase II is composed of 12 subunits, most of which are required for cell viability (1, 2). Two of these subunits, RPB4 and RPB7, can be dissociated as a complex from yeast RNA polymerase II when the enzyme is chromatographed on an anion exchange resin in the presence of 1.2–2 M urea (11–12) or when the enzyme is subjected to gel electrophoresis in the absence of a denaturing agent (i.e. SDS) (10). RNA polymerase II purified from some mutant strains of yeast is depleted of RPB4 and RPB7 subunits. These yeast mutants include *rpoB1* and *rpb4*, which contain a mutation in the largest subunit of RNA polymerase II and a deletion of the gene encoding RPB4, respectively (11, 31). Interestingly, a functional RPB7 gene is required for yeast cell viability (34), but cells lacking the RPB4 gene display a conditional phenotype (i.e. slow growth, increased sensitivity to heat and cold, and inositol auxotrophy) (31). The growth state and nutritional status of yeast cells has also been shown to influence the stoichiometry of RPB4/RPB7 subunits relative to other subunits in RNA polymerase II (35, 36). RNA polymerase II purified from yeast cells during logarithmic growth contains substoichiometric amounts of RPB4, but as stationary growth conditions are reached, the stoichiometry of this subunit approaches unity in the purified enzyme (36). Based on *in vitro* transcription experiments, RPB4 and RPB7 are not required for promoter-independent or nonspecific initiation and RNA chain elongation (11); however, these two subunits are required for promoter-dependent or specific initiation (12). These latter results have led to the hypothesis that RPB4 and RPB7 may function as initiation factors that shuttle reversibly on and off the enzyme (12).

It has been difficult to assess whether RNA polymerase II from other eukaryotic organisms contains subunits related to yeast RPB4 and RPB7 that reversibly dissociate and associate with the enzyme. Most plants, animals, and fission yeast lack an RNA polymerase subunit in the size class of yeast RPB4 (i.e. 32 kDa) with a mobility in SDS-PAGE between the large α-like subunit (i.e. RPB3 with a molecular mass of 36–45 kDa) and the largest subunit common to RNA polymerases I, II, and III (i.e. RPB5 with a molecular mass of 23–27 kDa). On the other hand, subunits of the same size class that share amino acid sequence similarity to yeast RPB7 have been identified in plant and animal RNA polymerase II (6, 13). In contrast to results with the yeast enzyme, results with wheat RNA polymerase II indicate that the RPB7-like subunit (RPB19.5) does not dissociate from the enzyme during native gel electrophoresis or electrophoresis carried out under mildly denaturing conditions (13).

The cloning of the *Arabidopsis* AtRPB15.9 subunit reported here has provided evidence for the existence of an RPB4-like subunit in plant RNA polymerase II, and sequence comparisons with EST data bases also suggest that an RPB4-like subunit exists in animal RNA polymerase II. Results presented here indicate the RPB4-like subunit in wheat and *Arabidopsis* RNA polymerase II is more stably associated with the enzyme than RPB4 in yeast RNA polymerase II and are consistent with previous results obtained with the wheat RPB7 subunit (13). These results include co-migration of plant RPB4 and/or RPB7 subunits with RNA polymerase II that is subjected to chromatography on DEAE-Sephadex with both a linear gradient and step elution in the presence of 1.2 or 2 M urea, chromatography on phosphocellulose with step elution in the presence of 2 M urea, and non-denaturing or mildly denaturing PAGE (13). Under the same conditions, reconstituted RPB4/RPB7 complexes display different chromatographic elution profiles than those displayed by plant RNA polymerase II. Taken together, our results with wheat and *Arabidopsis* RNA polymerase II indicate that the plant RPB4-like and RPB7-like subunits are more tightly associated with the enzyme than RPB4 and RPB7 in yeast RNA polymerase II. Whether the plant subunits shuttle on and off the enzyme and function as initiation factors in promoter-dependent transcription remains an open question. It is worth pointing out, however, that unlike the substoichiometric amounts of RPB4 and RPB7 that are generally found in yeast RNA polymerase II, the RPB4-like and RPB7-like subunits are present at approximately stoichiometric amounts in a
variety of RNA polymerase II enzymes purified from different plant tissues or cells exposed to a variety of conditions (i.e. quiescent germs, imbibed and growing germs, proliferating suspension culture cells, heat-shocked cells, inflorescences, and mitogenic hormone-treated tissues) (15–18, 37).

In addition to the difference in tenacity of RPB4-like and RPB7-like subunits for RNA polymerase II purified from yeast and plants, there is a major size difference between the yeast RPB4 subunit and the plant and animal RPB4-like subunits. The plant and animal subunits lack a highly charged 70 amino acid motif found in the center of yeast RPB4. This central motif and adjacent sequence have been reported to have some similarity to a motif in eubacterial σ factors (31), but the function, if any, of this motif in σ factors has not been reported. Because plant and animal RPB4-like subunits lack the central motif and any similarity to σ factors, the function of this central motif, if any, in transcription initiation in yeast remains to be determined. Based on in vitro interactions between recombinant Arabidopsis AtRPB15.9 and yeast RPB7 reported here and in vivo results with the human RPB7-like subunit and yeast RPB4 (6), it is unlikely that the central domain in yeast RPB4 is required for RPB4/RPB7 heterodimer formation. It is possible, however, that the highly charged central domain in yeast RPB4 accounts for the differences in stability of the RPB4/RPB7 complex in yeast versus plant RNA polymerase II.

The in vitro interaction results with recombinant RPB4-like and RPB7-like subunits indicate that these subunits from yeast and Arabidopsis form stable complexes, which are heterodimers. These in vitro interactions are not species-specific because mixed heterodimers are formed between Arabidopsis and yeast subunits (i.e. Arabidopsis RPB4/yeast RPB7 and yeast RPB4/Arabidopsis RPB7).

Identification of the RPB4-like subunit in plants and animals further categorizes the conserved nature of RNA polymerase II subunits in three eukaryotic kingdoms, plants, animals, and fungi. Sequences of cloned subunits and ESTs suggest that plant and animal RNA polymerase II enzymes contain 12 subunits that are related to the 12 subunits originally identified in yeast, S. cerevisiae. Many of the subunits from human have been shown to complement yeast subunits in strains defective in a specific RNA polymerase II subunit (4, 5).

In the future, it will be of interest to determine if any major differences exist for subunit function or assembly in RNA polymerase II from these three kingdoms of eukaryotes. It is not clear whether the RPB4-like and RPB7-like subunits in plants and animals shuttle on and off RNA polymerase II like the yeast RPB4/RPB7 complex and stimulate promoter-dependent transcription (12) or whether one or both of these subunits in plants and animals play a role in stress survival as reported for the yeast RPB4 subunit (35, 36).

REFERENCES
1. Thuriaux, P., and Sentenac, A. (1992) in The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression (Umes, E. W., Pringle, J. R., and Broach, J. R., eds) Vol. II, pp. 1–48, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
2. Woychik, N. A., and Young, R. A. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. C., and Conaway, J. W., eds) pp. 227–242, Raven Press, Ltd., New York.
3. McKune, K., and Woychik, N. A. (1994) Mol. Cell. Biol. 14, 4155–4159.
4. Shpakovski, G. V., Acker, J., Wintzerith, M., Lacroix, J. F., Thuriaux, P., and Vigneron, M. (1995) Mol. Cell. Biol. 15, 4702–4710.
5. McKune, K., Moore, P. A., Hull, M. W., and Woychik, N. A. (1995) Mol. Cell. Biol. 15, 6895–6900.
6. Khazak, V. S., Sadhale, P. P., Woychik, N. A., Brent, R., and Golemis, E. A. (1995) Mol. Biol. Cell. 6, 765–775.
7. Acker, J., de Graaff, M., Cheynel, I., Khazak, V., Kedinger, C., and Vigneron, M. (1997) J. Biol. Chem. 272, 16815–16821.
8. Umasov, T., Larkin, R. M., and Guilfoyle, T. J. (1996) J. Biol. Chem. 271, 5085–5094.
9. Sakurai, H., Miyao, T., and Ishihama, A. (1996) Gene 180, 63–67.
10. Delezee, S., Wyers, F., Sentenac, A., and Fromageot, P. (1976) Eur. J. Biochem. 65, 543–552.
11. Ruet, A., Sentenac, A., Fromageot, P., Winsor, B., and Lacroute, F. (1980) J. Biol. Chem. 255, 6450–6455.
12. Edwards, A. M., Kane, C. M., Young, R. A., and Kornberg, R. D. (1991) J. Biol. Chem. 266, 71–75.
13. Umasov, T., and Guilfoyle, T. J. (1992) J. Biol. Chem. 267, 23165–23169.
14. Lu, H., Flores, O., Weinmann, R., and Reindel, B. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10004–10008.
15. Jendrisak, J. J., and Burgess, R. R. (1977) Biochemistry 16, 1959–1964.
16. Guilfoyle, T. J., and Jendrisak, J. J. (1978) Biochemistry 17, 1860–1866.
17. Jendrisak, J. J., and Guilfoyle, T. J. (1978) Biochemistry 17, 1322–1327.
18. Guilfoyle, T. J., Hagen, G., and Malecm, S. (1984) J. Biol. Chem. 259, 640–648.
19. Regard, F., Bardet, C., Tremoussaye, D., Mioan, A., Lesure, B., and Alexa, M. (1993) FEBS Lett. 316, 153–156.
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (eds) (1996) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York.
22. Dellaporta, S. L., Woods, J., and Hicks, J. B. (1983) Plant Mol. Biol. 1, 19–21.
23. Doelling, J. H., and Pikaard, C. S. (1993) Plant Cell Rep. 12, 241–244.
24. Studier, F. W., and Moffatt, B. A. (1986) J. Mol. Biol. 189, 113–130.
25. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
26. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
27. Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 197–203.
28. Jendrisak, J. J., and Burgess, R. R. (1975) Biochemistry 14, 6439–6445.
29. Larkin, R. M., and Guilfoyle, T. J. (1997) J. Biol. Chem. 272, 12824–12830.
30. Stellwagen, E. (1990) Methods Enzymol. 182, 317–328.
31. Woychik, N. A., and Young, R. A. (1989) Mol. Cell. Biol. 9, 2854–2859.
32. Laemmli, U. K. (1970) Nature 227, 680–685.
33. Tang, H. Severinov, K., Goldfarb, A., and Elbricht, R. H. (1990) Proc. Natl. Acad. Sci. U. S. A. 92, 4902–4906.
34. McKune, K., Richards, K. L., Edwards, A. M., Young, R. A., and Woychik, N. A. (1993) Yeast 9, 295–299.
35. Choder, M. (1993) J. Bacteriol. 175, 6358–6363.
36. Choder, M., and Young, R. A. (1993) Mol. Cell. Biol. 13, 6984–6991.
37. Guilfoyle, T. J. (1983) in Enzymes of Nucleic Acid Synthesis and Modification (Jacob, S. T., ed) Vol. II, pp. 1–42, CRC Press, Inc., Boca Raton, FL.