Eukaryotic RNA Polymerases: Comparative Subunit Structures, Immunological Properties, and α-Amanitin Sensitivities of the Class II Enzymes from Higher Plants†

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ABSTRACT: The class II RNA polymerases from a phylogenetically diverse selection of higher plant species including four monocotyledonous species (hexaploid wheat, tetraploid wheat, rye, and maize) and two dicotyledonous species (soybean and cauliflower) were purified by Polymin P (polyethylenimine) fractionation, ammonium sulfate precipitation, DEAE-cellulose chromatography, phosphocellulose chromatography, and DNA-agarose chromatography. The subunit structures were compared by one-dimensional (urea and dodecyl sulfate) and two-dimensional (urea followed by dodecyl sulfate) polyacrylamide gel electrophoresis. The latter system was used to separate polypeptide subunits according to charge in the first dimension and molecular weight in the second dimension. Hexaploid wheat, tetraploid wheat, and rye class II RNA polymerases were indistinguishable under all conditions of electrophoresis and polypeptides with molecular weights and molar ratios of 220 000 (1), 140 000 (1), [42 000 + 40 000] (1), [27 000 + 25 000] (2), 21 000 (1), 20 000 (1), 17 800 (1), 17 000 (1), 16 300 (1), 16 000 (1), and 14 000 are associated with each of these purified enzymes. Maize RNA polymerase II contains polypeptides with the following molecular weights and molar ratios: 185 000 (1), 145 000 (1), 40 000 (1), [27 000 + 26 000] (2), 22 000 (1), 20 000 (1), 17 800 (1), 17 500 (1), 16 300 (1), 16 100 (1), and 14 000. Soybean RNA polymerase II contains polypeptides with the following molecular weights and molar ratios: 215 000 (1), 138 000 (1), 42 000 (1), 27 000 (2), 22 000 (1), 19 000 (1), 17 600 (1), 17 000 (1), 16 200 (1), 16 100 (1), and 14 000. Cauliflower RNA polymerase II contains polypeptides with the following molecular weights and molar ratios: 180 000 (1), 140 000 (1), 40 000 (1), 25 000 (2), 22 000 (1), 19 000 (1), 17 500 (1), 17 000 (1), 16 200 (1), 16 000 (1), and 14 000. A clear similarity among the subunit structures of the various plant class II RNA polymerases is evidenced by the number, molecular weights, and molar ratios of the polypeptides associated with each of the enzymes. This similarity extends to the charge densities of the subunits as revealed by two-dimensional polyacrylamide gel electrophoresis patterns, and analogous subunits can be identified in each enzyme. Antibodies directed against hexaploid wheat RNA polymerase II react with the enzymes purified from dicotyledonous plants as well as other monocotyledonous species, but they do not cross react with yeast RNA polymerase II or *Escherichia coli* RNA polymerase. All of the plant enzymes are 50% inhibited by approximately 0.05 μg/mL α-amanitin. These studies (1) indicate a highly conserved although enormously complex, molecular structure and (2) support the subunit status of the above polypeptides in RNA polymerase II from these higher plants.

Eukaryotic organisms possess three distinct classes of nuclear DNA-dependent RNA polymerase which transcribe different sequences of the nuclear genome (Roeder, 1976; Chambon, 1975). The three enzyme types, RNA polymerases I, II, and III, are each multisubunit proteins composed of two large polypeptides and several smaller polypeptides (Roeder, 1976; Chambon, 1975). Although putative subunit structures have been reported for these three major classes of nuclear RNA polymerase, recent results (Jendrisak & Burgess, 1977; Hodo & Blatti, 1977) suggest that the subunit structures may be more complex than originally reported.

Higher plant tissues contain high levels of soluble RNA polymerase II which can be purified to homogeneity by a routine procedure originally developed for the purification of wheat germ RNA polymerase II (Jendrisak & Burgess, 1975). We have used this method to purify milligram quantities of homogeneous RNA polymerase II from several higher plant species including hexaploid wheat, tetraploid wheat, rye, soybean, cauliflower, and maize.

In this study, we have compared the subunit structures of the class II RNA polymerases in these species by both one-dimensional (dodecyl sulfate or urea) and two-dimensional (urea followed by dodecyl sulfate) polyacrylamide gel electrophoresis. We have compared immunological similarities utilizing rabbit antibodies against wheat germ RNA polymerase II. In addition we have compared the α-amanitin sensitivities of RNA polymerase II from all of the above mentioned species.

We have shown that the subunit structures of RNA polymerase II from a variety of higher plants are equally as complex as wheat germ RNA polymerase II which has been extensively studied previously (Jendrisak & Burgess, 1977), and that a basic subunit structure is common to and is therefore highly conserved in both monocotyledonous and dicotyledonous plants. In addition, we have shown that these plant class II RNA polymerase enzymes have common antigenic determinants and similar α-amanitin inhibition properties.

Experimental Procedure

Materials. Wheat germs prepared from hexaploid wheat (*Triticum aestivum*) and tetraploid wheat (*T. durum*) were obtained from General Mills, Vallejo, Calif. Rye (*Secum cereale*) and maize (*Zea mays*) seed were obtained from the Minnesota Crop Improvement Association, St. Paul, Minn. Soybean (*Glycine max*) embryonic axes were obtained from Edible Soy Products, Hudson, Iowa. Cauliflower (*Brassica*

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oleracea var. *botrytis*) was obtained from a local market.

Embryos were prepared from rye seed by the method of Johnson & Stern (1957) which was originally developed for the preparation of wheat embryos. Soybean embryonic axes were purified by adjusting the density of a carbon tetrachloride–cyclohexane solution until contaminating cotyledonary tissue could be floated away from the embryonic axes. Maize seeds were germinated in moist vermiculite for four days in the dark at 30 °C. Twenty-four hours prior to the harvest of maize mesocotyl tissue, the etiolated seedlings were sprayed with a 2.5×10^{-3} M (pH 6.0) solution of 2,4-dichlorophenoxyacetic acid to induce nucleic acid synthesis in the mature tissue (West et al., 1960).

Acrylamide, methylenebisacrylamide, ammonium persulfate, tetraethylmethylenediamine, sodium dodecyl sulfate, Coomassie brilliant blue R-250, and agarose were purchased from Bio-Rad. Enzyme grade ammonium sulfate, sucrose and UltraPure urea were purchased from Schwarz/Mann. Tris[1] base, glycine, dithiothreitol, ethylenediaminetetraacetic acid, 2-mercaptoethanol, 2,4-dichlorophenoxyacetic acid, phenylmethanesulfonyl fluoride, and nucleoside triphosphates were purchased from Sigma. Ethylene glycol and glycerol were Baker Analyzed Reagents. DEAE-cellulose (DE-52) and phosphocellulose (P-11) were obtained from Whatman Polymin P was obtained from BASF (Rhein, Germany). [5-^{3}H]UTP (specific activity 18 Ci/mmol) was purchased from New England Nuclear. α-Amanitin was purchased from Henley and Co., New York, N.Y. Other chemicals were analytical reagent grade.

Buffers. Buffer A was 50 mM Tris-HCl (pH 7.9 at 4 °C), 250 mM sucrose, 1 mM $MgCl_2$, and 15 mM 2-mercaptoethanol. Immediately before use, 1/100 volume of 100 mM phenylmethanesulfonyl fluoride in 100% dimethyl sulfoxide was added to buffer A. Buffer B was 50 mM Tris-HCl (pH 7.9 at 4 °C), 75 mM ammonium sulfate, 1 mM ethylenediaminetetraacetic acid, and 15 mM 2-mercaptoethanol. Storage buffer was 50 mM Tris-HCl (pH 7.9 at 4 °C), 50% (v/v) glycerol, 1 mM dithiothreitol, and 1 mM ethylenediaminetetraacetic acid.

RNA Polymerase II Enzymes. RNA polymerase II was purified from 100-g amounts of wheat germ (hexaploid and tetraploid species), rye embryos and soybean embryonic axes, after homogenization in 5 volumes of buffer B, by Polymin P fractionation, ammonium sulfate precipitation, DEAE-cellulose chromatography, and phosphocellulose chromatography as described by Jendrisak & Burgess (1975). Cauliflower inflorescence and maize mesocotyl tissues (kg amounts) were first homogenized in 2 volumes of buffer A, the extracts were filtered through cheesecloth and Miracloth, and the chromatin was sedimented by centrifugation at 10 000*g* for 10 min. The class II RNA polymerases were then purified from the resulting supernatant solutions by the procedure described for embryonic tissues. DNA agarose chromatography was utilized as a final step of purification when necessary, to remove trace polypeptide contaminants (Guilfoyle & Jendrisak, 1978). DNA-agarose was prepared from wheat DNA (Jendrisak & Becker, 1973) by the procedure of Schaller et al. (1972). RNA polymerase activities were assayed throughout purification as described previously (Guilfoyle et al., 1975). Purified enzymes were concentrated by dialysis against storage buffer and were stored at −70 °C (Jendrisak & Burgess, 1977) where they were indefinitely stable. *Escherichia coli* RNA polymerase was purified by the method of Burgess & Jendrisak (1975). Yeast RNA polymerase II was purified by the Polymin P method used for purification of the plant enzymes.

Polyacrylamide Gel Electrophoresis. One-dimensional polyacrylamide gel electrophoresis in the presence of dodecyl sulfate was performed on 0.75-mm thick slab gels or 5-mm diameter cylindrical gels as described by Laemmli (1970). One-dimensional polyacrylamide gel electrophoresis in the presence of 8 M urea using a Tris–glycine buffer system was performed on 5-mm diameter cylindrical gels as described by Jovin et al. (1964). Two-dimensional polyacrylamide gel electrophoresis with the first dimension in 8 M urea and the second dimension in dodecyl sulfate was performed as described by Jendrisak & Burgess (1977). Polyacrylamide gels were stained with Coomassie brilliant blue and destained as described previously (Burgess & Jendrisak, 1975). Molecular weights of polypeptide subunits were derived from mobilities of standard proteins run on stacking dodecyl sulfate polyacrylamide gels (Jendrisak et al., 1976). Molar ratios of subunits were determined by densitometric scanning of Coomassie blue stained gels at 550 nm as described previously (Burgess & Jendrisak, 1975).

Immunological Techniques. Antibody prepared against hexaploid wheat germ RNA polymerase II in rabbits by the general methods described in Linn et al. (1973) was utilized to test the antigenic similarity of RNA polymerase II from the various plant species. The γ-globulin fraction was purified from sera (Linn et al., 1973). Double immunodiffusion was performed in 0.55% agarose containing 50 mM Tris-HCl (pH 7.9 at 25 °C), 100 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol. Two milliliters of agarose solution was poured into 3-cm diameter Petri dishes and wells of 3-mm diameter were punched after solidification of the agarose. The center well was filled with 25 μL of purified anti-wheat RNA polymerase II γ-globulins (6 mg/mL) and outer wells were filled with 25 μL of the appropriate RNA polymerase enzymes (approximately 10-μg amounts). Precipitation lines appeared after 24 to 48 h incubation at 4 °C.

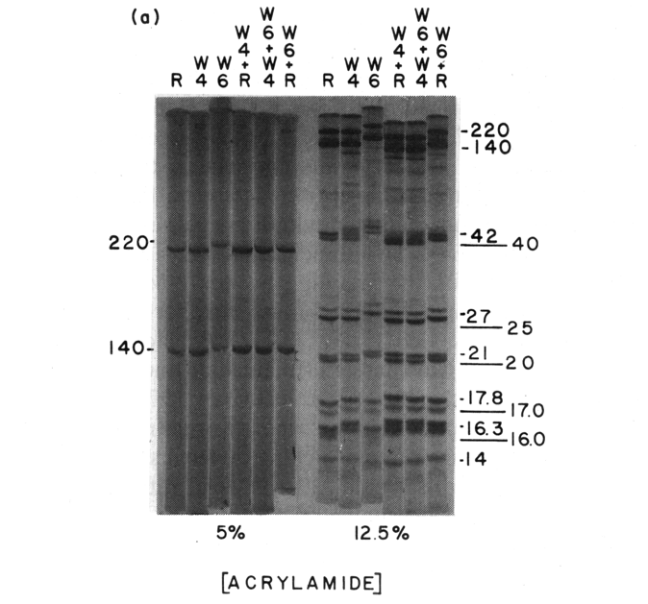
Results

The methodology introduced for the purification of wheat germ RNA polymerase II may be utilized for the routine purification of soluble RNA polymerase II from a variety of higher plants. An additional purification step, DNA–agarose chromatography, is sometimes required to remove contaminating polypeptides with molecular weights of approximately 100 000 (Guilfoyle & Jendrisak, 1978).

One-Dimensional Gel Electrophoresis in the Presence of Dodecyl Sulfate. Dodecyl sulfate polyacrylamide gel electrophoresis indicates that the purified class II RNA polymerases from three closely related monocotyledonous plant species, hexaploid wheat, tetraploid wheat and rye, are essentially identical in subunit structure (Figure 1a). Polypeptides (designated by letter) associated with the purified enzymes have the following molecular weights and molar ratios: (a) $220\,000_1$, (b) $140\,000_1$, $[(c_1)\ 42\,000 + (c_2)\ 40\,000]_1$, $[(d_1)\ 27\,000 + (d_2)\ 25\,000]_2$, (e) $21\,000_1$, (f) $20\,000_1$, (g) $17\,800_1$, (h) $17\,000_1$, (i) $16\,300_1$, (j) $16\,000_1$, and (k) 14 000. Molar ratios have been previously reported for hexaploid wheat RNA polymerase II (Jendrisak & Burgess, 1977) and similar subunit stoichiometry is observed in tetraploid wheat and rye RNA polymerase II. Molar ratios are presented as the nearest integral values which are accurate to within ±20%.

The subunit structure of RNA polymerase II from a more distantly related monocotyledonous plant, maize, is compared with wheat RNA polymerase II on a dodecyl sulfate–15% polyacrylamide gel (Figure 1b). The polypeptides associated with the maize enzyme have molecular weights and molar

[1] Abbreviation used: Tris, tris(hydroxymethyl)aminomethane.



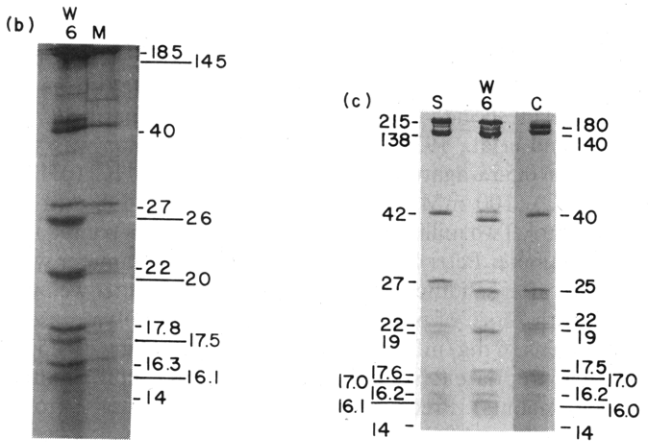


FIGURE 1: Polypeptide subunit patterns of hexaploid wheat (W6), tetraploid wheat (W4), rye (R), maize (M), soybean (S), and cauliflower (C) class II RNA polymerases displayed on dodecyl sulfate polyacrylamide gels. (a) Hexaploid wheat, tetraploid wheat and rye enzymes were run on 5-mm diameter cylindrical gels containing 5% and 12.5% polyacrylamide. Enzymes were run individually and as mixtures of two enzymes. Approximately 2 μg of each enzyme were applied to the 5% polyacrylamide gels and 10 μg of each RNA polymerase were applied to the 12.5% polyacrylamide gels. (b) Maize and hexaploid wheat RNA polymerase II enzymes (5 μg amounts) were run on a 0.75-mm thick, 15% polyacrylamide gel. (c) Soybean, cauliflower, and hexaploid wheat RNA polymerase II enzymes (10 μg amounts) were run on a 0.75-mm thick, 15% polyacrylamide gel. Migration was toward the anode from top to bottom. The numbers indicate approximate molecular weights of polypeptides in kilodaltons. See Table I for a summary of the subunit structure of each enzyme.

ratios of: (a′) 185 000_1, (b) 145 000_1, (c) 40 000_1, [(d_1) 27 000 + (d_2) 26 000]$_2$, (e) 22 000_1, (f) 20 000_1, (g) 17 800_1, (h) 17 500_1, (i) 16 300_1, (j) 16 100_1, and (k) 14 000, which are strikingly similar to the molecular weights and molar ratios of the polypeptides associated with wheat RNA polymerase II. However, the maize enzyme has a largest subunit (a′) of approximately 185 000 and has only a single polypeptide with a molecular weight near 40 000 in contrast to the doublet polypeptides observed with this approximate molecular weight in the wheat (hexaploid and tetraploid) and rye class II enzymes.

Figure 1c indicates that in both monocots and dicots the subunit structure of RNA polymerase II, as analyzed by one dimensional polyacrylamide gel electrophoresis in the presence of dodecyl sulfate, is highly conserved. Polypeptides with the following molecular weights and molar ratios are associated with soybean embryo RNA polymerase II: (a) 215 000_1, (b) 138 000_1, (c) 42 000_1, (d) 27 000_2, (e) 22 000_1, (f) 19 000_1, (g) 17 600_1, (h) 17 000_1, (i) 16 200_1, (j) 16 100_1, and (k) 14 000. Polypeptides with the following molecular weights and molar ratios are associated with the cauliflower inflorescence RNA polymerase II: (a′) 180 000_1, (b) 140 000_1, (c) 40 000_1, (d) 25 000_2, (e) 22 000_1, (f) 19 000_1, (g) 17 500_1, (h) 17 000_1, (i) 16 200_1, (j) 16 000_1, and (k) 14 000. The dicot class II RNA polymerases have single polypeptides at 40 000 and 25 000, in contrast to the doublet polypeptides with these approximate molecular weights in the wheat (hexaploid and tetraploid) and rye enzymes.

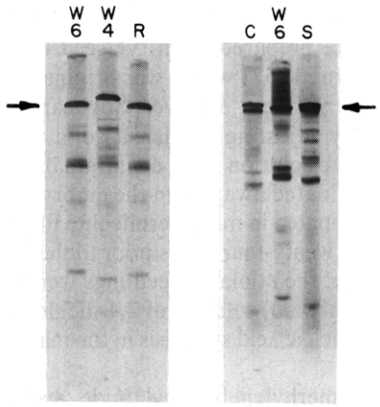


FIGURE 2: Polypeptide subunit patterns of hexaploid wheat (W6), tetraploid wheat (W4), rye (R), soybean (S), and cauliflower (C) class II RNA polymerases displayed on 7.5% polyacrylamide gels containing 8 M urea at pH 8.7. Approximately 20 μg of each enzyme were applied to the gels. Migration was toward the anode from top to bottom. The arrows indicate the interface between the stacking and separating gels.

A general subunit structure for RNA polymerase II from plants emerges from this study. All enzymes analyzed have two high molecular weight subunits of approximately 220 000 and 140 000 or 180 000 and 140 000. In addition, all enzymes have a subunit (a molar ratio of 1) or two subunits (an additive molar ratio of 1) of approximately 40 000; a subunit (a molar ratio of 2) or two subunits (an additive molar ratio of 2) of about 27 000; two subunits of about 20 000 (a molar ratio of 1 for each polypeptide); two subunits of approximately 17 000 (a molar ratio of 1 for each polypeptide); two subunits of about 16 000 (a molar ratio of approximately 1 for each polypeptide); and a subunit of approximately 14 000 of variable stoichiometry, usually less than a molar ratio of 1. Molecular weights of putative subunits of RNA polymerase II enzymes from each of the plant species analyzed are summarized in Table I.

One-Dimensional Polyacrylamide Gel Electrophoresis in the Presence of 8 M Urea at pH 8.7. While polyacrylamide gel electrophoresis in the presence of dodecyl sulfate shows that the molecular weights of the polypeptides associated with RNA polymerase II from a number of higher plants are highly conserved, this analysis fails to indicate whether the inherent charges of presumably analogous polypeptides are likewise conserved. To evaluate the conservation in charge density for each polypeptide associated with the plant class II enzymes, we have utilized polyacrylamide gel electrophoresis in the presence of 8 M urea in a Tris–glycine buffer system, which separates polypeptides largely on the basis of charge. Figure 2 shows that, on the basis of charge, hexaploid and tetraploid wheat and rye RNA polymerase II subunit structures are essentially identical. Enzymes from soybean and cauliflower give similar, although not identical, polypeptide patterns when compared with wheat on 8 M urea gels (Figure 2). It is inter-

TABLE I: Subunit Structures of Plant RNA Polymerase II Enzymes.

Subunit	Subunit mol wt × 10^{-3}					
	Wheat (6*n*)	Wheat (4*n*)	Rye	Soybean	Cauliflower	Maize
a	220	220	220	215		
a′					180	185
b	140	140	140	138	140	145
c	42, 40	42, 40	42, 40	42	40	40
d	27, 25	27, 25	27, 25	27	25	27, 26
e	21	21	21	22	22	22
f	20	20	20	19	19	20
g	17.8	17.8	17.8	17.6	17.5	17.8
h	17.0	17.0	17.0	17.0	17.0	17.5
i	16.3	16.3	16.3	16.2	16.2	16.3
j	16.0	16.0	16.0	16.1	16.0	16.1
k	14	14	14	14	14	14

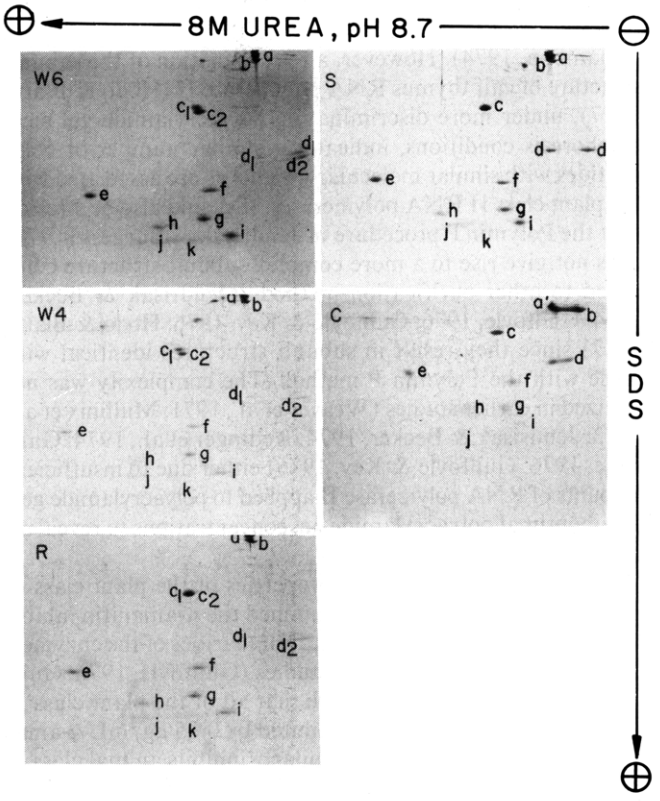


FIGURE 3: Polypeptide subunit patterns of hexaploid wheat (W6), tetraploid wheat (W4), rye (R), soybean (S), and cauliflower (C) class II RNA polymerases displayed on a two-dimensional polyacrylamide gel electrophoresis system. The first dimension (horizontal) consisted of electrophoresis of individual enzymes on 7.5% polyacrylamide cylindrical gels containing 8 M urea at pH 8.7 (see Figure 2), and the second dimension (vertical) consisted of electrophoresis of polypeptides out of the urea gels onto 3-mm thick, 15% polyacrylamide slab gels containing dodecyl sulfate. Approximately 25 μg of each enzyme were subjected to analysis. Directions of migration and gel systems are indicated by the arrows. Letters correspond to the polypeptides listed in Table I.

esting to note that only in the case of cauliflower does a high molecular weight subunit enter the resolving gel. This apparently results from the loss by proteolytic cleavage of a basically charged polypeptide segment associated with the 220 000 polypeptide but absent in the 180 000 polypeptide.

Two-Dimensional Polyacrylamide Gel Electrophoresis. To identify which polypeptides seen on 8 M urea gels correspond to those observed on dodecyl sulfate gels, we used two-dimensional gel electrophoresis with the first dimension in the presence of 8 M urea and the second dimension in the presence of dodecyl sulfate. The two-dimensional gel patterns for hexaploid and tetraploid wheat, rye, soybean, and cauliflower are shown in Figure 3. The nearly identical two-dimensional gel electrophoretic patterns show that the polypeptides with molecular weights near 25 000 are basic in charge since they do not migrate into the stacking gel in the first dimension. One of the two 20 000 polypeptides in each enzyme is relatively acidic to the other low molecular weight (less than 45 000) RNA polymerase II subunits since it migrates with the marker dye in the first dimension. The remaining low molecular weight polypeptides are somewhat intermediate in charge. The high molecular weight subunits (220 000 and 140 000) fail to enter the separating gel in the first dimension. In each case, the polypeptide patterns for each of the plant class II RNA polymerases derived from two-dimensional gel electrophoresis are highly similar to one another. These results indicate a conservation of not only molecular weights but of the inherent charges of enzyme subunits. These results also allow us to assign an unambiguous identification of analogous subunits in all of the enzymes (Table I).

Immunological Studies. The antigenic similarity of the various plant RNA polymerase II enzymes was tested by double immunodiffusion using antibodies prepared against hexaploid wheat germ RNA polymerase II (Figure 4). Although all of the plant class II enzymes tested gave precipitation lines, the lines were much stronger for hexaploid and tetraploid wheat and rye than for the dicotyledonous species, soybean and cauliflower. Yeast RNA polymerase II and *E. coli* RNA polymerase did not cross react with antibodies for wheat RNA polymerase II.

α-Amanitin Sensitivities. The α-amanitin titration profiles of the purified class II enzymes are shown in Figure 5. In each case, the plant class II enzymes are 50% inhibited by a concentration of approximately 0.05 μg/mL α-amanitin.

Discussion

This study indicates that higher plant RNA polymerase II enzymes are highly complex in subunit structure, but that this subunit structure is highly conserved in both monocotyledonous and dicotyledonous species. A generalized subunit structure for the plant class II enzymes emerges from this study. In each case, the enzymes are composed of: two large subunits (220 000 and 140 000 or 180 000 and 140 000); a single or doublet subunit(s) of approximately 40 000; a single or doublet subunit(s) of approximately 27 000 which is/are positive in charge; two subunits of approximately 20 000, one subunit of

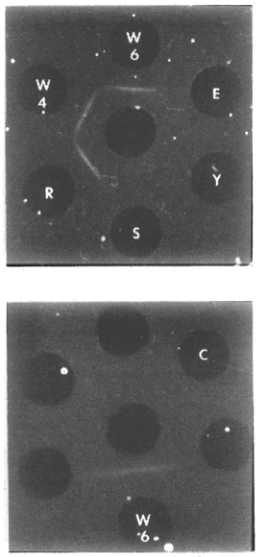


FIGURE 4: Double immunodiffusion (Ouchterlony) analysis of hexaploid wheat (W6), tetraploid wheat (W4), rye (R), yeast (Y), cauliflower (C), and soybean (S) class II RNA polymerases and *E. coli* RNA polymerase (E). The center wells contained 150 μg of anti-wheat RNA polymerase II γ-globulins in 25 μL and the outer wells contained approximately 10 μg of the indicated enzymes.

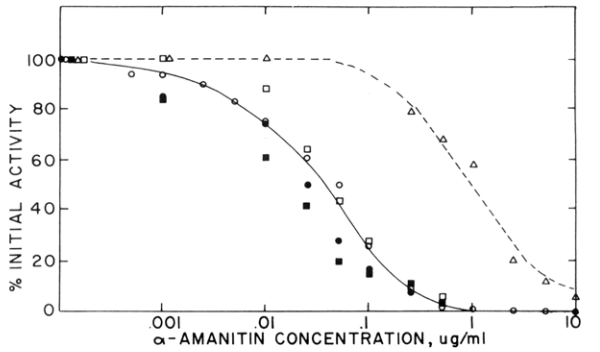


FIGURE 5: α-Amanitin sensitivities of class II RNA polymerases from hexaploid wheat (○), soybean (●), cauliflower (□), maize (■), and yeast (△). Each enzyme was assayed in the presence of various concentrations of α-amanitin.

which is highly negatively charged at pH 8.7; two subunits of approximately 17 000; two subunits of approximately 16 000; and a single polypeptide of approximately 14 000. This highly conserved and complex molecular structure of these class II enzymes suggests that these polypeptides may all be required for transcription of the plant genome. The doublet subunits of approximately 40 000 and 25 000 in the wheat and rye enzymes may reflect protein modification or expression of multiple subunit alleles in these species.

An interesting characteristic of the class II enzymes from higher plant tissues is that greater than 90% of the enzyme activity is not pelleted with nuclear or chromatin fractions (Guilfoyle et al., 1975; Jendrisak & Burgess, 1975), but is present in a readily soluble form. This property is of substantial aid in the purification of the enzymes since they apparently do not have to be dissociated from a nucleoprotein complex. We find little variation in the class II RNA polymerase subunit structures in different plant species or different tissues of the same plant. The notable exception to this observation concerns the highest molecular weight subunit in the enzymes. RNA polymerase II from ungerminated embryonic plant tissues is largely form IIA RNA polymerase since the largest subunit has a molecular weight in excess of 200 000. In contrast, actively growing (germinated) tissues contain form IIB RNA polymerase with a largest subunit of 180 000. Rye, tetraploid and hexaploid wheat, and soybean class II RNA polymerases were purified from dormant (ungerminated) embryos and are form IIA enzymes. The maize and cauliflower class II RNA polymerases were purified from nondormant (growing) tissues and are form IIB enzymes. Recently we have also shown that IIB RNA polymerase predominates in 5 day old soybean hypocotyl (actively growing) tissue (Guilfoyle & Jendrisak, 1978). The presence of different forms of RNA polymerase II in ungerminated and actively growing plant tissues suggests that the germinating plant embryo may be a very useful system for investigating the significance of multiple forms of RNA polymerase II.

Two-dimensional polyacrylamide gel electrophoresis used in this study has provided a clear picture of the similarities in subunit structure of the variety of enzymes tested here. Results indicate a conservation in molecular weight and charge of subunits. These subunit structures appear to be more complex than the structures of RNA polymerase II from animal tissues (Chambon, 1974). However, a reexamination of the subunit structure of calf thymus RNA polymerase II (Hodo & Blatti, 1977), under more discriminating polyacrylamide gel electrophoresis conditions, indicates a similar number of polypeptides with similar molecular weights as are associated with the plant class II RNA polymerases. It should also be stressed that the Polymin P procedure of Jendrisak & Burgess (1975) does not give rise to a more complex subunit structure compared to other purification methods (Jendrisak & Becker, 1974; Guilfoyle, 1976; Guilfoyle & Key, 1976; Hodo & Blatti, 1977) since they result in subunit structures identical with those with the Polymin P method. The complexity was not realized in earlier studies (Weaver et al., 1971; Mullinix et al., 1973; Jendrisak & Becker, 1974; Kedinger et al., 1974; Guilfoyle, 1976; Guilfoyle & Key, 1976) either due to insufficient amounts of RNA polymerase II applied to polyacrylamide gels or suboptimal polyacrylamide gel concentrations to reveal all of the smaller polypeptides.

In addition to the subunit properties of the plant class II RNA polymerases, we have examined the α-amanitin inhibition properties and immunological similarities of the enzymes. In agreement with our earlier studies (Guilfoyle, 1976; Guilfoyle & Key, 1976), we observe that all of the plant class II RNA polymerases are 50% inhibited by 0.05 μg/mL α-amanitin, a concentration which similarly inhibits animal class II RNA polymerase. To assure accuracy in this determination, the concentration of α-amanitin was determined spectrophotometrically from the extinction coefficient $E_{305nm}^{1M} = 15\,400$ (Cochet-Meihlac & Chambon, 1974). We have also included an α-amanitin inhibition curve of yeast RNA polymerase II (Figure 5) and we obtain 50% inhibition at approximately 1 μg/mL α-amanitin as reported previously by other investigators (Schultz & Hall, 1976). Our results are in marked contrast to results on α-amanitin sensitivity of wheat germ RNA polymerase II reported by Hodo & Blatti (1977). The reason for the low degree of α-amanitin sensitivity (50% inhibition at 0.271 μg/mL) reported in the latter study may have been due to inaccurate measurement of α-amanitin concentrations or the use of excessively high concentrations of wheat germ RNA polymerase II in the assays.

The dicot class II RNA polymerases clearly possess antigenic determinants in common with monocot enzymes as revealed by this study. This was not unexpected in light of the fact that antibodies against mammalian class II RNA polymerase react with cognate enzymes of nonmammalian origin (Ingles, 1973).

The studies presented here substantiate a basic subunit structure of plant class II RNA polymerases since apparently analogous subunits have similar molecular weights, molar ratios and charge densities. The subunit status of the polypeptides found to be associated with each of the purified enzymes is supported by the following evidence: (a) a similar subunit structure can be found for RNA polymerase II from a wide variety of plant species; (b) we find no evidence of fractionation of these purified enzymes on a variety of ion exchange or gel filtration chromatographic resins; (c) the polypeptide molar ratios in each column fraction are constant after a variety of chromatographic steps; and (d) one major band is observed after gel electrophoresis of the purified enzymes under nondenaturing conditions (Jendrisak et al., 1976). The structures are complex but highly conserved as seen by analysis of dicot and monocot enzymes. The reason for this complexity is presently unknown but the fact that large amounts of plant enzymes can be routinely prepared homogenous from various plant species at various developmental stages may provide insight toward understanding the complexity of RNA polymerase II subunit structure.

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References

Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14,* 4634.

Chambon, P. (1974) *Enzymes, 3rd Ed. 10,* 261.

Chambon, P. (1975) *Annu. Rev. Biochem. 43,* 613.

Cochet-Meihlac, M., & Chambon, P. (1974) *Biochim. Biophys. Acta 353,* 160.

Guilfoyle, T. J. (1976) *Plant Physiol. 58,* 453.

Guifoyle, T. J., & Jendrisak, J. J. (1978) *Biochemistry* (in press).

Guilfoyle, T. J., & Key, J. L. (1976) in *Molecular Mechanisms in the Control of Gene Expression* (Nierlich, D. P., & Rutter, W. M., Eds.) pp 267–272, Academic Press, New York, N.Y.

Guilfoyle, T. J., Lin, C. Y., Chen, Y., Nagao, R. T., & Key, J. L. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 69.

Hodo, H. G., & Blatti, S. P. (1977) *Biochemistry 16,* 2334.

Ingles, C. J. (1973) *Biochem. Biophys. Res. Commun. 55,* 364.

Jendrisak, J. J., & Becker, W. M. (1973) *Biochim. Biophys. Acta 319,* 48.

Jendrisak, J. J., & Becker, W. M. (1974) *Biochem. J. 139,* 771.

Jendrisak, J. J., & Burgess, R. R. (1975) *Biochemistry 14,* 4639.

Jendrisak, J. J., & Burgess, R. R. (1977) *Biochemistry 16,* 1959.

Jendrisak, J. J., Petranyi, P. W., & Burgess, R. R. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 779–791, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Johnson, F. B., & Stern, H. (1957) *Nature (London) 179,* 160.

Jovin, T., Chrambach, A., & Naughton, M. A. (1964) *Anal. Biochem. 9,* 351.

Kedinger, C., Gissinger, F., & Chambon, P. (1974) *Eur. J. Biochem. 44,* 421.

Laemmli, U. K. (1970) *Nature (London) 227,* 680.

Linn, T. G., Greenleaf, A. L., Shorenstein, R. G., & Losick, R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 1865.

Mullinix, K. P., Strain, G. C., & Bogorad, L. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70,* 2386.

Roeder, R. G. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 285–329, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Schaller, H., Nusslein, C., Bonhoeffer, F. J., Kurtz, C., & Nietzschmann, I. (1972) *Eur. J. Biochem. 26,* 474.

Schultz, L. D., & Hall, B. D. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 1029.

Weaver, R. F., Blatti, S. P., & Rutter, W. J. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68,* 2994.

West, S. H., Hanson, J. B., & Key, J. L. (1960) *Weeds 8,* 333.