THE SUBUNIT STRUCTURES OF SOLUBLE AND CHROMATIN-BOUND RNA POLYMERASE II FROM SOYBEAN

Tom J. Guilfoyle[1] and Joe L. Key
Departments of Botany and Biochemistry
University of Georgia
Athens, Georgia 30602

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SUMMARY: DNA-dependent RNA polymerase II is present in two forms, IIa and IIb, in germinating soybean. Form IIa is the dominant form of the enzyme in ungerminated embryos and appears to be a soluble enzyme. Form IIb increases in amount as germination progresses and is tightly bound to the chromatin template. The subunit structures of soybean RNA polymerases IIa and IIb are identical except for the molecular weights of their largest subunits which are 200,000 daltons and 170,000 daltons for IIa and IIb, respectively. The enzymes have seven common subunits: 142,000, 42,000, 26,000, 20,000, 16,000, 15,500, and 14,000 daltons.

INTRODUCTION

DNA-dependent RNA polymerase II is the enzyme responsible for the synthesis of HnRNA and presumably mRNA in eukaryotes (1, 2). This enzyme exists in two or three forms (3, 4) which differ only in the molecular weights of their largest subunits. The dominant forms of the enzyme, IIa and IIb, possess largest subunits of approximately 200,000 and 170,000 daltons, respectively (3, 4). The relative levels of RNA polymerase IIa and IIb vary in different organisms (5, 6), in different tissues of the same organism (6), and during development of the organism (7). By choosing the appropriate isolation conditions and the appropriate developmental stage of the tissue, soybean RNA polymerases IIa and IIb can be purified and characterized independently from one another. RNA polymerase IIa is the dominant form (greater than 95%) of the Class II enzyme in ungerminated soybean embryos and is a soluble enzyme. Little if any RNA polymerase II is associated with chromatin or nuclei in ungerminated embryos. With the

[1]Present Address: Department of Botany, University of Minnesota, St. Paul, Minnesota, 55108

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onset of germination, increasing amounts of RNA polymerase II can be recovered in nuclear preparations as a chromatin-bound enzyme which actively synthesizes RNA in vitro in isolated nuclei and chromatin. The chromatin-bound Class II enzyme exists exclusively as the Form IIb enzyme. The soluble Form IIa enzyme may represent a storage or precursor form of RNA polymerase II which becomes active only after its conversion via proteolytic cleavage to the Form IIb enzyme.

MATERIALS AND METHODS

Soybean embryonic axes were dissected from the dry seeds of Glycine max, variety Wayne. RNA polymerase II was purified from soybean embryos by the method of Jendrisak and Burgess (8) as modified by Guilfoyle and Key (9). Soybean nuclei and chromatin were prepared with a pH 6 buffer as described previously (10, 11). RNA polymerase II was solubilized from chromatin and purified by the method described by Guilfoyle and Key (9). The procedures of Laemmli (12) were used for gel electrophoresis with the exception that stacking gels were not utilized. Gels were fixed, stained with Coomassie Brilliant Blue, and destained as described by Valenzuela et al. (13). All chemicals used in electrophoresis were from BioRad.

RESULTS AND DISCUSSION

Soybean embryonic axes, similar to wheat germ (8, 14), are a rich source of RNA polymerase IIa. As with wheat germ (8, 14), the soybean Class IIa enzyme is a soluble enzyme, and little if any RNA polymerase II activity is associated with nuclei or chromatin in ungerminated soybean embryos (Guilfoyle, unpublished). With the onset of germination, soybean nuclei exhibit an increased level of RNA polymerase II activity (Guilfoyle, unpublished) which is tightly bound to the chromatin template. The RNA polymerase II activity can be dissociated from the chromatin template by sonication at relatively high ionic strength (15). Purification of the soluble and chromatin-bound RNA polymerase II enzymes from soybean results in the subunit patterns shown in Figure 1. RNA polymerase IIa (the soluble enzyme) and IIb (the chromatin-bound enzyme) have largest subunits of 200,000 (a) and 170,000 (b) daltons, respectively. Both RNA polymerase IIa and IIb have common subunits of (c) 142,000, (d) 42,000, (e) 26,000,

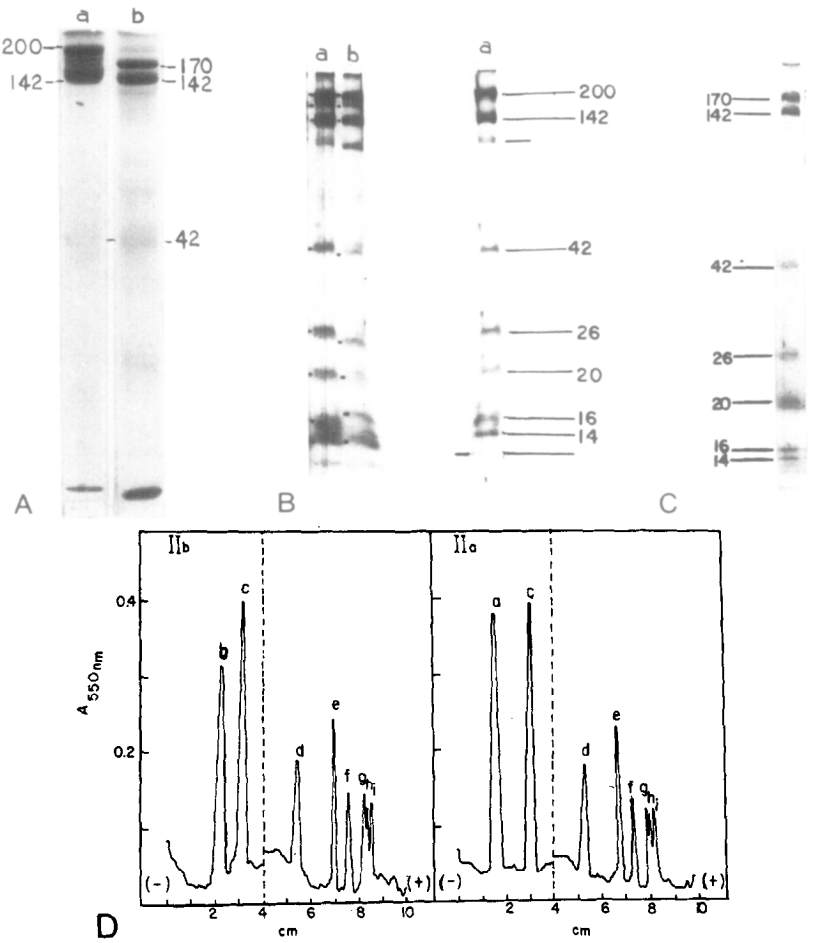

Figure 1. Putative subunit structures of soluble (IIa) and chromatin-bound (IIb) RNA polymerase II enzymes. Molecular weights of the polypeptides are given in kilodaltons. (A) Soluble (a) and chromatin-bound (b) RNA polymerase II from soybean electrophoresed on 10% SDS polyacrylamide cylindrical gels. (B) Soluble soybean (a) and wheat germ (b) RNA polymerase IIa electrophoresed on 8.75 - 15% SDS polyacrylamide linear gradient slab gels. The unlabeled polypeptide is a contaminant which occurs in variable amounts. (C) Chromatin-bound RNA polymerase IIb from soybean electrophoresed on 8.75 - 15% SDS polyacrylamide linear gradient slab gels. (D) SDS polyacrylamide gel scans of soybean RNA polymerase IIb and IIa. The cylindrical gels contained two percentages of polyacrylamide (indicated by the dashed line) with the upper half being 7.5% and the lower half 15% polyacrylamide. Gels were scanned at 550 nm with a linear transport attachment to a Gilford spectrophotometer. Putative subunits are labeled a-i and correspond to molecular weights of a.) 200,000, b.) 170,000, c.) 142,000, d.) 42,000, e.) 26,000, f.) 20,000, g.) 16,000, h.) 15,500, and i.) 14,000 daltons.

(f) 20,000, (g) 16,000, (h) 15,500, and (i) 14,000 daltons. The soluble enzyme (IIa) from soybean is strikingly similar in subunit composition to the soluble Class II enzyme purified from wheat germ (14). In both cases, the dominant form of the Class II enzyme is IIa. In marked contrast, the subunit structure of chromatin-bound RNA polymerase II from soybean is nearly identical to the chromatin-bound RNA polymerase II purified from cauliflower inflorescence nuclei (15). The chromatin-bound enzyme in each case is a Form IIb enzyme. Thus it may be a general characteristic (at least in higher plants) that RNA polymerase IIa is a soluble, extranuclear enzyme while RNA polymerase IIb is a chromatin-bound enzyme which is active in the transcription of mRNAs. Initial characterization of the purified Class IIa and IIb enzymes has revealed that the enzymes have identical divalent cation optima, ionic strength optima, and template requirements (Guilfoyle, unpublished).

A partitioning of soluble (IIa) and chromatin-bound (IIb) RNA polymerase II within animal nuclei has also been reported (16), but subunit analyses of these enzymes were not presented. The more rigorous procedures required for the isolation of plant nuclei compared to animal nuclei may result in the selective loss of any soluble Form IIa enzyme being associated with plant nuclei. Although the possibility exists that the Class IIb enzyme could be artifactually generated by the proteolytic cleavage of the largest subunit of RNA polymerase IIa during the purification procedure, our studies suggest that the distribution of the enzymes within the cell may be of physiological significance. The presence or absence of protease inhibitors (i.e., PMSF) during the purification procedures has no influence on the subunit patterns observed with the Form IIa or IIb enzymes in soybean, wheat germ, or cauliflower. Although recent evidence suggests that yeast RNA polymerase IIb is derived from IIa via proteolytic cleavage (17), this proteolysis is inhibited by the protease inhibitor, PMSF. Other evidence indicates that the conversion

of animal Form IIa enzyme to Form IIb enzyme during routine purification procedures is minimal and does not significantly influence the relative levels of RNA polymerase IIa and IIb observed in specific tissues (6). The significance of the studies with the plant Class II enzymes is that the RNA polymerase IIb may be the form of RNA polymerase II which is active in transcription, and RNA polymerase IIa may represent a storage or precursor enzyme which is proteolytically cleaved before it becomes active on chromatin.

Soybean embryos and wheat germ offer unique systems to study the postulated storage or precursor role for RNA polymerase IIa (or IIo, see Schwartz and Roeder, 4) and its conversion into an actively transcribing form, IIb. Both systems are extremely rich in RNA polymerase IIa (8, 9) in their quiescent or ungerminated state. The Form IIa enzyme is entirely soluble and the Form IIb enzyme is essentially absent from the ungerminated embryos. With the onset of germination, mRNA synthesis (18) and nuclear associated RNA polymerase II activity are rapidly activated (Guilfoyle, unpublished). In soybean, this nuclear associated RNA polymerase II activity has been shown to be exclusively Form IIb enzyme. Antibodies to the RNA polymerase II enzymes and their largest subunits will facilitate a more rigorous examination of the roles and intracellular distribution proposed for Class IIa and IIb enzymes in germinating plant tissue.

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