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Peptide maps comparing subunits of maize chloroplast and type II nuclear DNA-dependent RNA polymerases

(RNA nucleotidyltransferase/*Zea mays*)

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ABSTRACT **Both one- and two-dimensional peptide mapping techniques have been used to compare the 180-kilodalton subunits from maize chloroplast and type II nuclear DNA-dependent RNA polymerases (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6). Despite their similar molecular mass, these 180-kilodalton subunits are not the same. Also, two-dimensional tryptic maps of the 160-, 43-, and 28-kilodalton polypeptides from maize type II nuclear RNA polymerase and of the 140-, 42-, and 27-kilodalton polypeptides from maize chloroplast RNA polymerase show that each of these six polypeptides is unique.**

Cells of eukaryotic green plants contain at least three separate genomes. DNA is found together with DNA-dependent RNA polymerases (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) in their chloroplasts and mitochondria as well as in their nuclei (1–4). The maintenance and development of each organelle, as well as of the cell as a whole, require the participation of all three genomes. For example, ribosomes and other multimeric components of organelles are composed of organelle- and nuclear-coded elements (4). So, not only does RNA synthesis have to be regulated within each organelle, but also gene expression in such multigenomic cells must be coordinated and integrated. Intracellular integration of transcription could be effected through functional ties between nuclear and organellar RNA polymerases.

Chloroplast and type II nuclear RNA polymerases have been prepared from subcellular fractions of maize leaf homogenates (5–14). Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of these two maize RNA polymerases has shown that each enzyme contains two large subunits which are present at all stages of purification. The molecular masses of these polypeptides are 180 and 160 kilodaltons (kDal) for the type II nuclear RNA polymerase (12, 14, 15) and 180 and 140 kDal for the chloroplast enzyme (11). Moreover, when a sample containing both the plastid and nuclear enzymes is electrophoresed through a single denaturing gel, the 180-kDal subunits of the two RNA polymerases are not resolved (12). Intracellular-intergenomic integration of transcription via sharing of a common subunit by chloroplast and type II nuclear RNA polymerases seems an appealing possibility.

In this paper we compare, by one- and two-dimensional mapping, proteolytic fragments of the 180-kDal subunits as well as other pairs of polypeptides from maize chloroplast and type II nuclear RNA polymerases. None of the pairs of subunits of the two enzymes resembles one another.

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MATERIALS AND METHODS

Plants. Maize seeds (*Zea mays*, FR9^c ms × FR37, Illinois Foundation Seeds) were soaked in water, then sown in moist vermiculite, and grown in a greenhouse for 7–9 days with supplemental light.

RNA Polymerases and Subunits. Chloroplast RNA polymerase was solubilized from isolated maize chloroplasts (7) and was purified by glycerol gradient centrifugation and phosphocellulose chromatography (11, 13). Type II nuclear RNA polymerase was purified as described (10, 12) with modifications (14).

Individual subunits were obtained from purified RNA polymerases by sodium dodecyl sulfate/polyacrylamide gel electrophoresis in slabs composed of a 5% (wt/vol) stacking gel and a 10% (wt/vol) separating gel (16). Electrophoresis was at 15 mA for 1 hr and then at 25 mA for the remaining time in the apparatus devised by Studier (17). Gels were stained with Coomassie brilliant blue R and then destained by the method of Cleveland *et al.* (18). Electrophoretic patterns of the maize chloroplast and type II nuclear enzymes were identical to those presented elsewhere (10–15).

Single, discrete, Coomassie blue-stained polypeptide bands were sliced from slab gels with a razor blade and were subjected to peptide mapping.

Peptide Maps. Polypeptides were analyzed by two different techniques. Two-dimensional tryptic mapping of ^{125}I-labeled polypeptides was performed as described in the modification by Kidd *et al.* (19) of the procedure of Elder *et al.* (20). One-dimensional peptide mapping, involving limited proteolysis in sodium dodecyl sulfate, was carried out by the method of Cleveland *et al.* (18) with modifications (21).

RESULTS AND DISCUSSION

The one- and two-dimensional peptide mapping techniques we used have been shown to be useful in detecting structural relationships among different polypeptides (19–23). In Fig. 1, the two-dimensional tryptic map of the 180-kDal polypeptide from purified maize chloroplast RNA polymerase is compared with that of the 180-kDal polypeptide associated with maize nuclear RNA polymerase II. The two-dimensional tryptic maps of these two subunits were clearly different. However, because of the large numbers of tryptic peptides produced from such large subunits, some regions of the two-dimensional maps contained unresolved fragments. Consequently, we subjected both polypeptides to one-dimensional peptide mapping by

Abbreviation: kDal, kilodalton.

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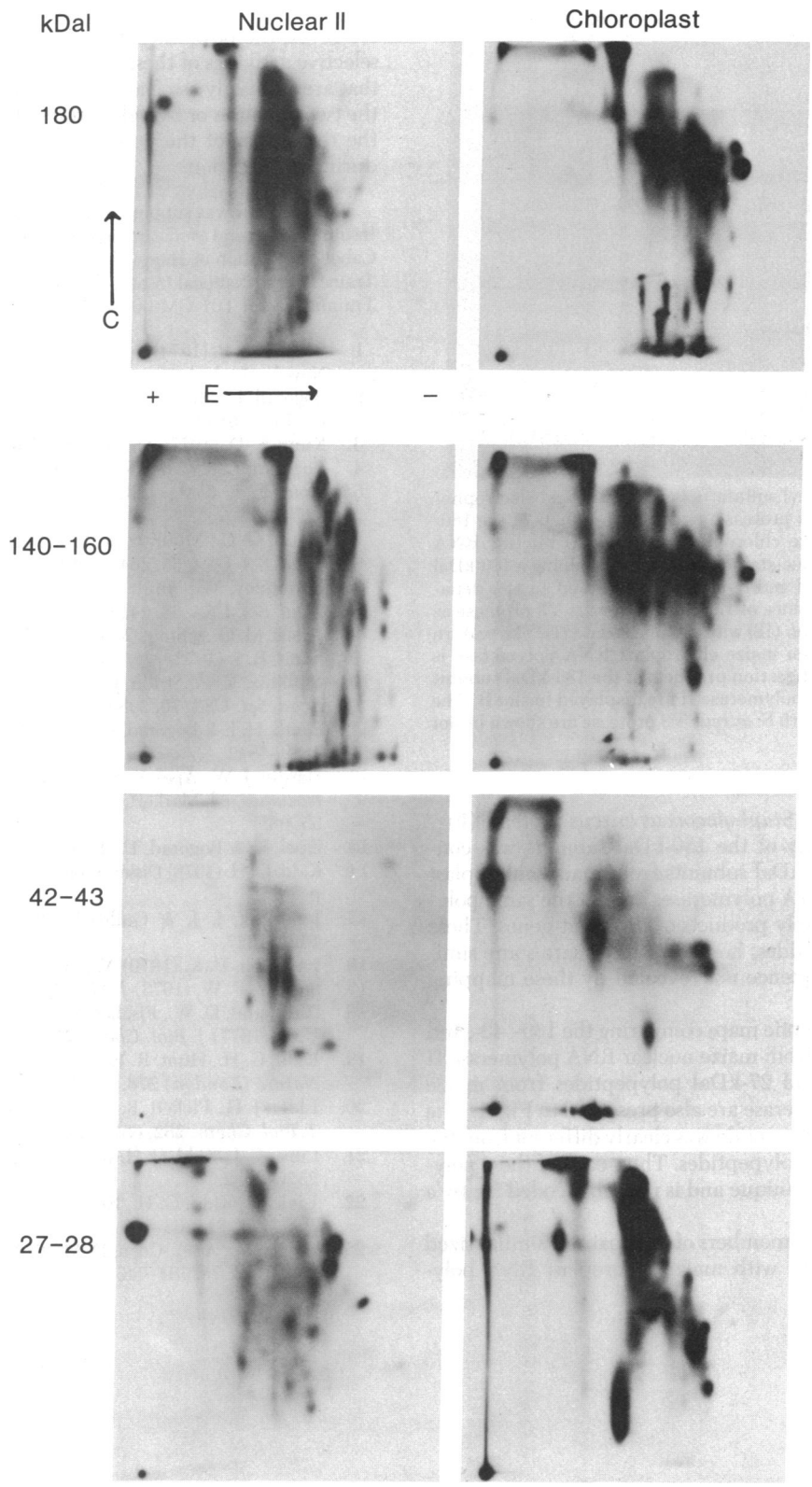

FIG. 1. Comparison of the ^{125}I-labeled tryptic peptides of subunits from maize type II nuclear and chloroplast RNA polymerases. Polypeptides were labeled with ^{125}I and digested with trypsin. The tryptic peptides were resolved in two dimensions and visualized by autoradiography (19). The directions of electrophoresis (E) and chromatography (C) are indicated at the origin on the map of the 180-kDal subunit from maize nuclear RNA polymerase II. Maize nuclear RNA polymerase II contains polypeptides of 180, 160, 43, and 28 kDal, whereas maize chloroplast RNA polymerase has polypeptides of 180, 140, 42, and 27 kDal. Besides these sets of similar-sized subunits, additional polypeptides of other sizes are associated with each enzyme (2, 10, 11, 12, 14).

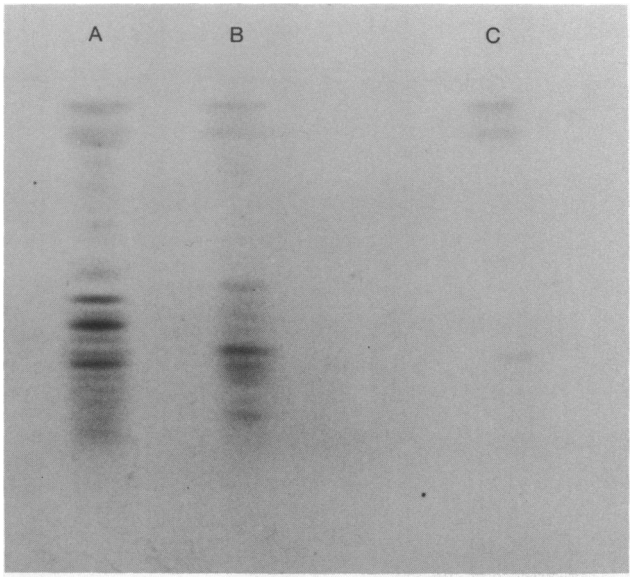

FIG. 2. Sodium dodecyl sulfate/polyacrylamide gel electrophoretic patterns of *S. aureus* protease-generated products of the 180-kDal subunits from maize chloroplast and type II nuclear RNA polymerases. Coomassie blue-stained gel slices, containing a 180-kDal subunit, were equilibrated and re-electrophoresed on an 18% denaturing slab gel in the presence of 1.0 μg of *S. aureus* V8 protease as described by Cleveland *et al.* (18) with modifications (21). The pattern of digestion products from maize chloroplast RNA polymerase is displayed in slot A. The digestion products of the 180-kDal subunit from maize nuclear RNA polymerase II are displayed in slot B. The polypeptides associated with *S. aureus* V8 protease are shown in slot C.

limited proteolysis with *Staphylococcus aureus* protease (Fig. 2), and the dissimilarity of the 180-kDal subunits was confirmed. Thus, the 180-kDal subunits from maize chloroplast and type II nuclear RNA polymerases are not the same polypeptide and are probably products of different genes. These two 180-kDal polypeptides, however, may share some similarities in primary sequence not revealed by these mapping methods.

Two-dimensional tryptic maps comparing the 160-, 43-, and 28-kDal polypeptides from maize nuclear RNA polymerase II with the 140-, 42-, and 27-kDal polypeptides from maize chloroplast RNA polymerase are also presented in Fig. 1. The tryptic map of each polypeptide was clearly different from the maps of the five other polypeptides. Thus, each of these polypeptides appears to be unique and is probably coded for by a different gene.

These data show that members of four pairs of similar sized polypeptides associated with maize chloroplast RNA polymerase are unrelated in primary structure based on comparison of proteolytic fragments. That these two enzymes have distinctive properties (6, 7, 10, 11) is thus confirmed and extended. However, the possibility is not excluded that coordination of selective activities of these enzymes is exerted by polypeptides that are primarily associated with DNA sequences common to the two genomes or by polypeptides that interact loosely with the remainder of the polymerase and are consequently lost during purification.

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