NO EVIDENCE FOR TISSUE-SPECIFIC SEQUENCES OF CYTOPLASMIC 5 S AND 5.8 S RIBOSOMAL RNA's IN THE BROAD BEAN

P.I. PAYNE, J. WOLEDGE* and M.J. CORRY

Agricultural Research Council Unit of Developmental Botany, 181A Huntingdon Road, Cambridge CB3 ODY, U.K.

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1. Introduction

It has recently been reported that *Xenopus laevis* 5 S rRNA is heterogeneous [1, 2]. There is at least one sequence of 5 S rRNA which is restricted to ovaries, whereas another sequence is common to kidney cells, liver, spleen and testis as well as ovaries. Heterogeneity is consistent with the finding of multiple copies of DNA cistrons which code for 5 S rRNA in *Xenopus* [3] and a variety of other eukaryotic organisms [4, 5]. It is of interest to know if heterogeneity of 5 S rRNA occurs in other eukaryotic organisms and to determine if tissue-specific sequences of other RNA species which are synthesized from multiple DNA cistrons do occur. We have examined the primary structures of 5 S and 5.8 S rRNA's in the leaves, stems and roots of the broad bean (*Vicia faba* var. White Fan), but have found no evidence for tissue-specific sequences.

2. Methods

Root 5 S and 5.8 S rRNA's were labelled with ^{32}P by keeping 8–12 day-old seedlings with their roots immersed in 100 ml of distilled water containing 80 μg/ml chloramphenicol and 100 μCi/ml of [^{32}P]-orthophosphate for 48 hr. RNA was extracted by the naphthalene 1,5-disulphonate-phenol method as previously described [6] and then dissolved in 0.65 M NaCl–0.025 M potassium acetate buffer (pH 5.6 at 20°C). The pellet obtained after centrifugation for 3×10^7 g_{av} min [ref. 6] was dissolved in 0.5% (w/v) sodium dodecyl sulphate–0.15 M sodium acetate buffer (pH 6.0) and 5.8 S RNA released from high molecular weight RNA by heating and rapidly cooling [7]. The RNA was fractionated by electrophoresis through a slab of 10% polyacrylamide gel [8] with a 2.6% spacer gel and 5.8 S RNA was detected by autoradiography. The supernatant obtained by high-speed centrifugation was mixed with 2 vol of absolute ethanol to precipitate the RNA. 5 S RNA was isolated by electrophoresis in cylinders of 12.5% polyacrylamide gel [8] with a 2.6% spacer. Gels were scanned at 260 nm using a modified Hilger–Gilford spectrophotometer. Both 5 S and 5.8 S RNA fractions were recovered from gels by homogenisation in 0.2 M NaCl–0.025 M potassium acetate buffer (pH 5.6 at 20°C), followed by purification in 2 cm × 0.4 cm columns of DEAE-cellulose [9].

Stem and leaf 5 S and 5.8 S RNA's were labelled with ^{32}P by inserting surface-sterilized shoots of dark grown plants into 20 ml of water containing 500 μCi/ml [^{32}P]orthophosphate for 48 hr under high light intensity to allow uptake in the transpiration stream. Cytoplasmic 5 S (freed of chloroplast 5 S RNA) and 5.8 S rRNA's were extracted exactly as described for roots.

The primary structure of RNA fractions was investigated using the fingerprinting procedures of Sanger and Brownlee [10].

3. Results and discussion

An autoradiograph of a complete T_1-ribonuclease

* Permanent address: Grassland Research Institute, Hurley, Maidenhead, Berkshire, SL6 5LR, U.K.

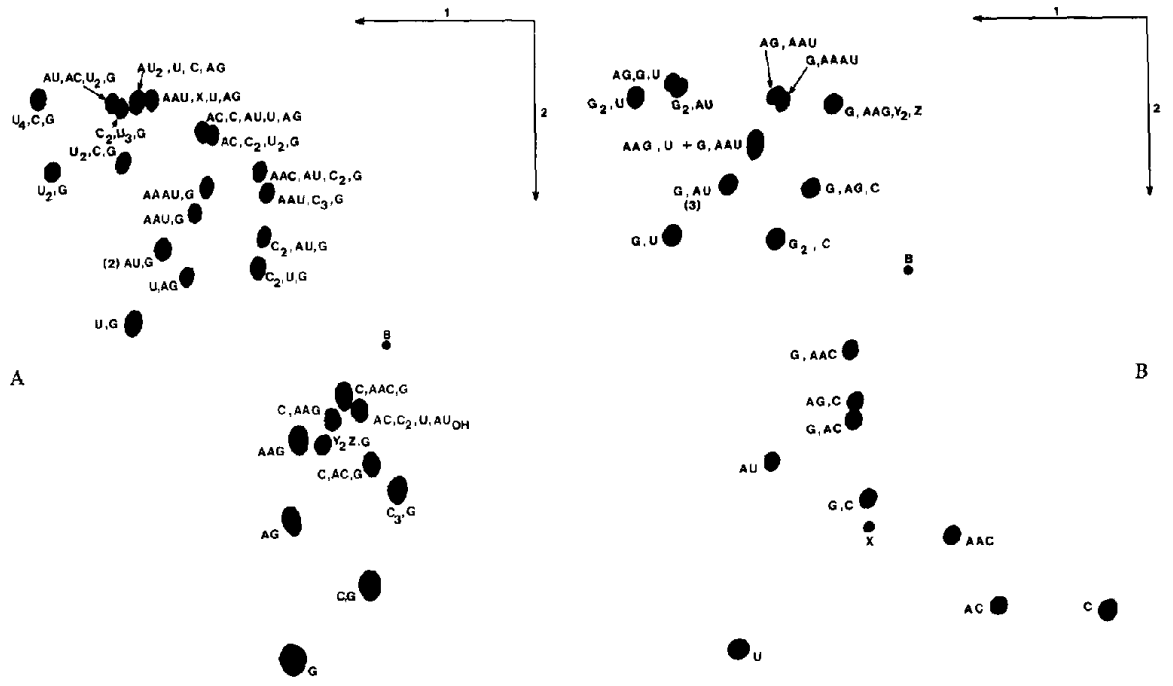

Fig. 1. Diagram showing the position of nucleotides from a complete ribonuclease T_1 digest (A) and a complete pancreatic ribonuclease A digest (B) of broad bean root 5.8 S rRNA after fractionation by ionophoresis in cellulose acetate at pH 3.5: 1) followed by DEAE-cellulose paper in 7% formic acid; 2) [ref. 10]. Secondary digestion products are given for each fraction. Figures in brackets represent molar yields when greater than unity, oligonucleotides occurring in submolar quantities have been omitted. X, Y and Z are probably minor bases. B indicates the position of the blue dye (xylene cyanol F.F.). The 3' end group $-U_{OH}$ was determined using snake venom phosphodiesterase and alkaline hydrolysis [11]. A 5' end group could not be found.

fingerprint of 5.8 S RNA from roots is shown in fig. 1A. The end groups and each fragment larger than a dinucleotide were quantified for radioactivity by scintillation spectrometry and analysed further by digestion with pancreatic A ribonuclease [10]. The fingerprints of 5.8 S rRNA from stems and leaves were identical to those of root 5.8 S rRNA; the oligonucleotides were present in similar yields and gave the same products upon digestion with pancreatic A ribonuclease. The three 5.8 S RNA species were also fingerprinted after complete digestion with pancreatic A ribonuclease (see fig. 1B) and each fragment was completely digested with ribonuclease T_1. Again no sequence differences could be detected.

The T_1 and pancreatic A ribonuclease fingerprints of root 5 S RNA (fig. 2 A and B) are quite different from the corresponding fingerprints of 5.8 S RNA. But again, T_1 and pancreatic A fingerprints of 5 S rRNA from roots, stems and leaves are very similar and oligonucleotides gave identical products when digested with pancreatic A and T_1-ribonuclease respectively.

In addition to those oligonucleotides which occur in 5 S and 5.8 S RNA in molar, bimolar and trimolar quantities, there are some present in fingerprints (not shown in figs. 1 and 2) with molar yields of 0.1–0.3. These could arise from: 1) minor heterogeneity in the 5 S and 5.8 S RNA's; 2) contamination by rRNA breakdown products; 3) contamination by 4 S (transfer) RNA and 4) RNA of fungal or bacterial origin. Although we get clear separations of 5.8 S, 5 S and 4 S RNA's in our gels [see ref. 9] and can therefore eliminate possibility 3), we cannot distinguish between possibilities 1), 2) and 4).

There are certain restrictions in the methods we have used to distinguish between two sequences. For

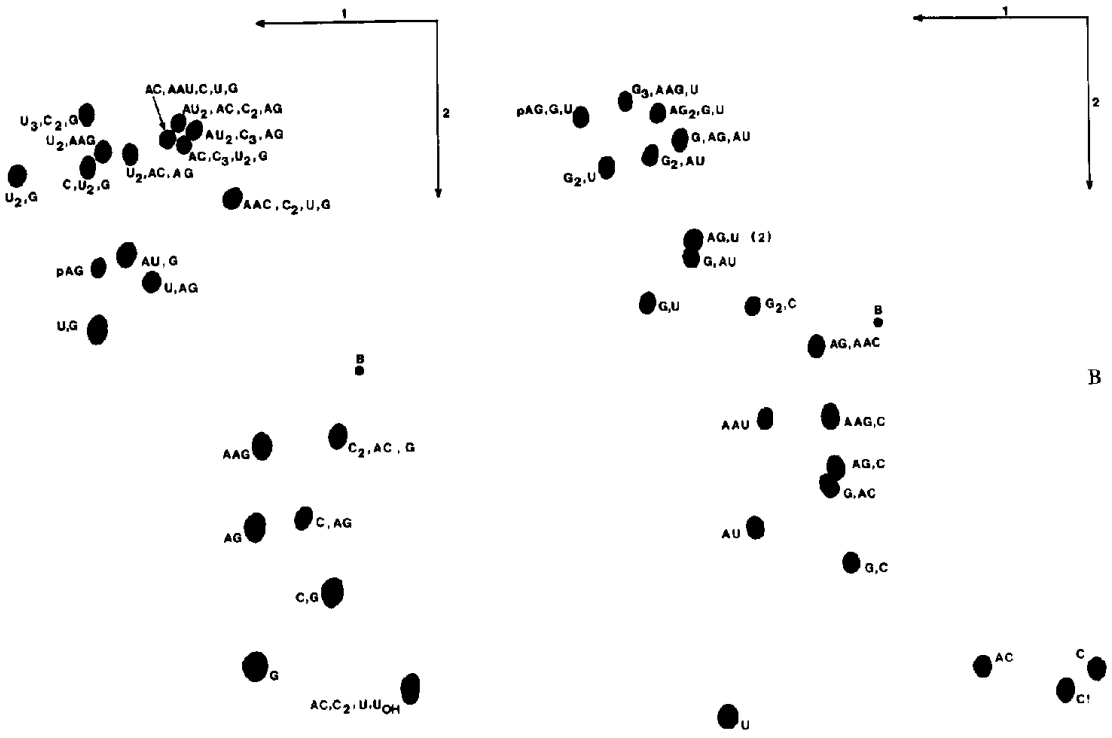

Fig. 2. Diagram showing the position of nucleotides from a complete ribonuclease T_1 digest (A) and a complete pancreatic ribonuclease A digest (B) of broad bean root 5 S rRNA after the two dimensional fractionation procedure described in the legend of fig. 1. The 5′ end group pA– was determined using alkaline hydrolysis and alkaline phosphatase digestion followed by alkaline hydrolysis [11]. C! represents cytidine 2′,3′ cyclic monophosphate.

example, two changes in a single ribonuclease T_1 oligonucleotide may pass undetected provided they are compensating as exemplified by C–C–A–C–U–C–Gp and C–U–A–C–C–C–Gp. Statistically, it is very unlikely that a compensating substitution of this type will occur if there are no detectable single changes. Analysis of pancreatic A ribonuclease oligonucleotides further reduces the number of undetected complementary substitutions.

To conclude, within the limitations of the procedures used, we have found no evidence for tissue-specific sequences of 5 S and 5.8 S rRNA in the broad bean.

This work forms part of a programme designed to determine the complete nucleotide sequences of broad bean cytoplasmic and chloroplast 5 S RNA's.

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