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ORIGIN OF 7s RIBOSOMAL RNA IN ARTICHOKE

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Key Word Index—*Helianthus tuberosus*; Compositae; artichoke tubers; ribosomal RNA; 7s *r*RNA; *r*RNA processing; *r*RNA cistrons; tissue culture; polyacrylamide gel electrophoresis.

Abstract—Total nucleic acids were prepared from cultured explants of artichoke tuber tissue labelled with ^{32}P for 1·5 hr and fractionated by electrophoresis on polyacrylamide gels. A comparison of native and denatured *r*RNA profiles showed that after denaturation, the $1{\cdot}33 \times 10^6$ dalton (25s) moiety is resolved into two units, one of $1{\cdot}28 \times 10^6$ daltons and the other of 5×10^4 daltons (7s RNA). These two molecules, which are covalently bonded together in the $1{\cdot}39 \times 10^6$ daltons precursor to 25s *r*RNA are cleaved during the final stage of processing of the *r*RNA gene transcript to the mature 25s *r*RNA. A comparison of the specific activities of the $1{\cdot}28 \times 10^6$ and 5×10^4 dalton molecules over a range of labelling times indicates that the latter occurs at the 3′ end of the $1{\cdot}39 \times 10^6$ daltons precursor of the 25s moiety.

INTRODUCTION

Native ribosomal RNA from the large subunit of all 80S ribosomes (25–28s *r*RNA) contains two *r*RNA components which are held together by hydrogen bonds [1], the smaller of these being the 7s [2] or 5·8s [3] *r*RNA. The mature *r*RNAs (25–28s and 18s) are transcribed as one covalently bonded molecule 20–50% larger in size than the sum of the sizes of the mature *r*RNA molecules. This initial transcription product is then processed by specific nuclease cleavage and digestion to produce the two mature *r*RNAs (25–28s and 18s), the discarded portions of the chain (the transcribed spacer) being broken down in the nucleus [4–6]. The studies of Pène *et al.* [2] on HeLa *r*RNA suggested that the 7s *r*RNA was part of this initial transcript and was released by the final nuclease processing step in the maturation of the 28s *r*RNA molecule. Limited nuclease digestion has confirmed that the 7s *r*RNA is covalently bonded to the 28s *r*RNA in the 32s precursor to HeLa 28s *r*RNA [7]. The present work was designed to show whether the final step in the maturation of artichoke 25s *r*RNA ($1{\cdot}33 \times 10^6$ daltons) involves the cleavage of the 7s molecule from the $1{\cdot}39 \times 10^6$ dalton precursor or whether the 7s is cleaved from the mature 25s *r*RNA molecule some time later, by determining whether the 25s *r*RNA ever exists as a single covalently bonded chain and whether, by examining the relative labelling kinetics of the 7s *r*RNA and 25s *r*RNA from which the 7s molecule has been removed by denaturation, the position of the 7s molecule in the precursor could be found.

RESULTS AND DISCUSSION

Figure 1 shows the release of the 7s molecule by 5 min incubation at 60° in electrophoresis buffer. The size of the 7s as determined by its mobility compared with those of the 4s and 5s ($2{\cdot}5 \times 10^4$ and $3{\cdot}79 \times 10^4$ daltons respectively) is 5×10^4 daltons, comparable with the size of the 7s in other plant species [1].

Figure 2a shows the E_{265} and radioactivity profiles of a total native preparation from tissue incubated with ^{32}Pi. The radioactivity peak associated with the E_{265} 25s *r*RNA is a combined peak of $1{\cdot}33 \times 10^6$ (the mature 25s *r*RNA) and $1{\cdot}39 \times 10^6$

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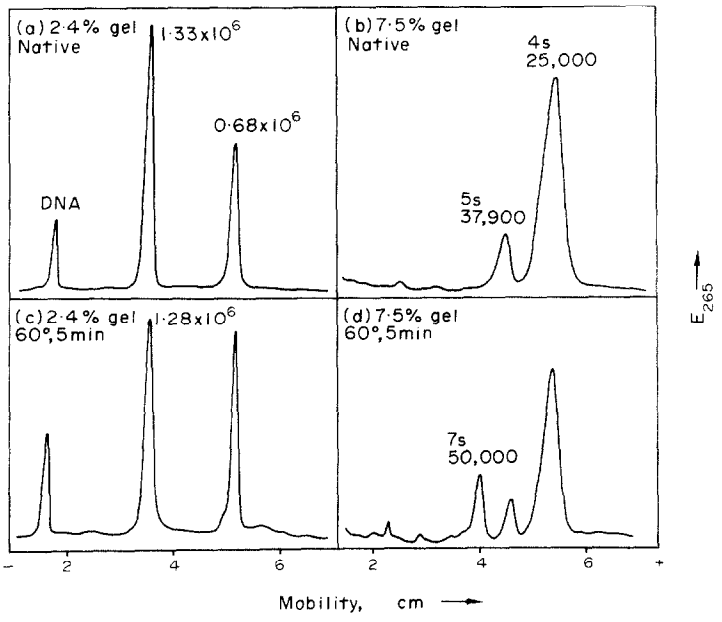

Fig. 1. Release of 7s RNA by thermal denaturation. Artichoke nucleic acid was fractionated by electrophoresis on 2·4% (a and c) or 7·5% (b and d) polyacrylamide gels for 3 hr either in a native state (a and b) or after incubation at 60° for 5 min (c and d).

dalton (its immediate precursor) components. The electrophoretic profile also shows the initial transcription product of the *r*RNA gene ($2{\cdot}5 \times 10^6$ daltons) moving just ahead of the DNA and the intermediate precursor of the $0{\cdot}68 \times 10^6$ dalton stable *r*RNA (18s) at $0{\cdot}9 \times 10^6$ daltons. When this preparation is heated at 60° for 5 min prior to fractionation, the combined peak splits clearly into two (Fig. 2b). The 25s precursor persists with a MW of $1{\cdot}39 \times 10^6$ daltons, while a new component appears corresponding to a MW of $1{\cdot}28 \times 10^6$ daltons and there appears to be no intermediate radioactive peak corresponding to the $1{\cdot}33 \times 10^6$ daltons molecule indicating that this has lost a 7s component. To confirm this, fractions from the combined peak in Fig. 2a were isolated, heated at 60° for 5 min and re-electrophoresed. The heavy side of the double peak (slice 3, Fig. 2c) appears to show only the $1{\cdot}39 \times 10^6$ dalton precursor and the light side (slice 7, Fig. 2e) only the $1{\cdot}28 \times 10^6$ dalton mature peak less the 7s, while a fraction from the middle of the combined peak (slice 5, Fig. 2d) shows both the $1{\cdot}39 \times 10^6$ and $1{\cdot}28 \times 10^6$ dalton peaks with no apparent trace of a discrete component of an intermediate size. Slices 5 and 7 (from the middle and light side of the native peak) would be expected to contain a measureable percentage of $1{\cdot}33 \times 10^6$ dalton *r*RNA relative to the $1{\cdot}28 \times 10^6$ dalton component if the former does exist for anytime as a covalently bonded chain since both molecules in the native state, before the 7s is removed, would have a MW of $1{\cdot}33 \times 10^6$ daltons. The gel analyses, however, show that the $1{\cdot}28 \times 10^6$ dalton peak is symmetrical indicating that if the $1{\cdot}33 \times 10^6$ dalton component does exist as a covalently bonded molecule, it must be considerably less stable than any of the other known *r*RNA precursors. Thermal denaturation thus appears to have converted all of the $1{\cdot}33 \times 10^6$ dalton *r*RNA to the $1{\cdot}28 \times 10^6$ dalton molecule, indicating that the former, observed in the native state, does not exist as a covalently bonded unit. The final processing stage which cleaves the 25s *r*RNA from the $1{\cdot}39 \times 10^6$ dalton precursor therefore involves the cleavage of $1{\cdot}1 \times 10^5$ daltons of polynucleotide, only 5×10^4 daltons of which can be accounted for by the 7s molecule. No evidence was found for a $1{\cdot}1 \times 10^5$ dalton fragment which was further cleaved to give the 7s molecule. This suggests that the $1{\cdot}1 \times 10^5$ dalton component is rapidly cleaved again to yield the 7s which remains hydrogen bonded to the $1{\cdot}28 \times 10^6$

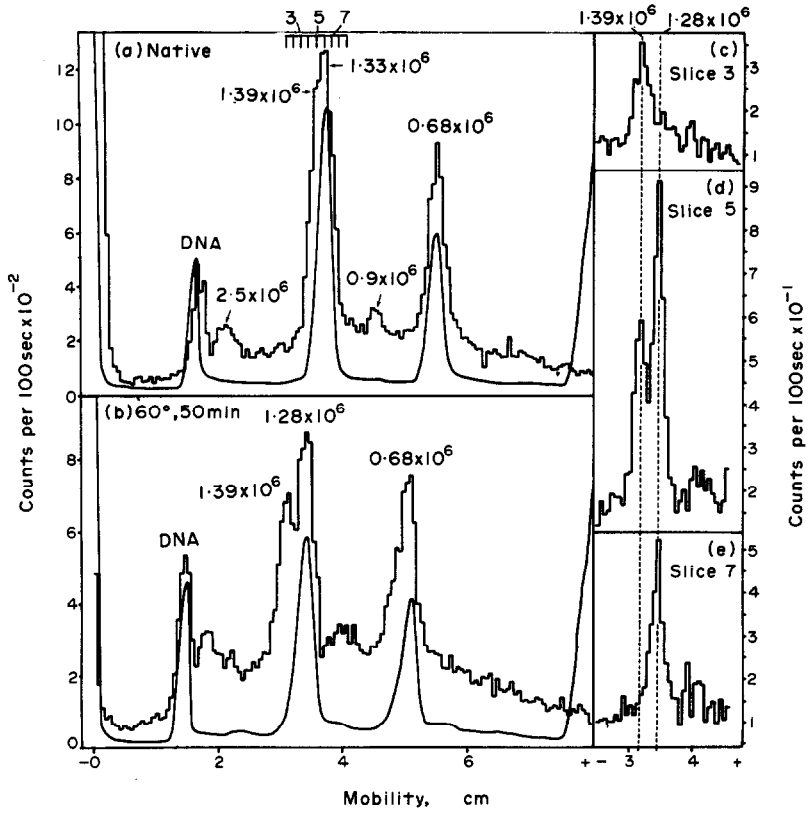

Fig. 2. Thermal denaturation profiles of ^{32}Pi labelled artichoke *r*RNA. Artichoke tissue was labelled with ^{32}Pi for 1·5 hr and the nucleic acid was fractionated by electrophoresis on 2·4% polyacrylamide gels for 2·5 hr (a) native, (b) after incubation at 60° for 5 min, (c, d, e) show the effect of 5 min denaturation at 60° on the RNA in slices taken from the combined 1·39 × 10^6 and 1·33 × 10^6 dalton radioactive peak as indicated in (a), (c) slice 3, (d) slice 5, (e) slice 7. Continuous line: A_{265}, histogram: ^{32}Pi.

dalton *r*RNA molecule and a 6 × 10^4 dalton fragment which must be rapidly degraded since no evidence for a fragment of this size was found. A comparison of the sp. act. of the 1·28 × 10^6 dalton and 7s components after relatively short labelling periods should indicate the intramolecular location of the 7s unit, since the first radioactivity to appear in the completed molecules will be that incorporated as the terminal nucleotides at the 3′ end of the 1·39 × 10^6 precursor. Measurements could not be made with incubations shorter than 50 min because of insufficient incorporation, but after 50–

Table 1. Specific activities of 1·28 × 10^6 and 5 × 10^4 daltons *r*RNA species

Duration of label	Ratio of sp. act. 5 × 10^4 *r*RNA/1·28 × 10^6 *r*RNA		Approximate sp. act. (cpm/μg)
	Experiment 1	Experiment 2	
50 min		2·38	100
60 min	2·84	1·95	350
75 min	1·30		550
6 hr		1·06	4390

^{32}Pi labelled artichoke nucleic acid was prepared, and purified as described in Experimental. Specific activities were determined from the A_{265} and ^{32}Pi profiles of nucleic acid samples incubated at 60° for 5 min and fractionated on gels as in Fig. 1 c and d.

60 min the sp. act. of the 7s was up to 3 times greater than that of the $1{\cdot}28 \times 10^6$ dalton molecule (Table 1). The ratio decreased, and after 6 hr was 1·06. The very large sp. act. ratios which might be expected at the shortest labelling times were not detectable because of insufficient incorporation and the gradient of uptake and incorporation through the explant tissue. The higher sp. act. of the 7s molecule at the shorter times however, indicates that it is located at the 3′ end of the immediate precursor to the 25s *r*RNA.

In *Xenopus*, it has been shown by hybridization and equilibrium density centrifugation studies [8] that the 7s gene lies between the 18 and 28s *r*RNA cistrons as part of the transcribed unit. If a similar situation holds for artichoke, the results presented here would indicate that the artichoke 25s *r*RNA cistron is at the 5′ end of the transcribed unit as has been shown for HeLa by direct electron microscopic analysis of the precursor [6] and for Novikoff Hepatoma Ascites cells by 5′ terminal sequence analysis of the precursor and the mature 28s *r*RNA [9], rather than at the 3′ end as suggested for HeLa from cordycepin [10] and for *Xenopus* from *in vitro* transcription studies [11].

EXPERIMENTAL

Artichoke tuber tissue (*Helianthus tuberosus* cv Bunyard's Round) was excised under sterile conditions and cultured in 250 ml roller bottles (75 explants per 7·5 ml, 2·5 rpm) [12] for 72–96 hr to ensure asynchronous cell division and growth. The culture was then labelled with ^{32}Pi (33 μCi/ml, 5 Ci/mg P) for various periods of time and total nucleic acid purified [13] and fractionated by electrophoresis on 8 cm 2·4 or 7·5% polyacrylamide gels essentially as described by Loening [14]. Gels were scanned at 265 nm. Distribution of radioactive RNA was determined by freezing gels in dry ice to the scanned length prior to cutting into 0·5 mm slices. Slices were either dried onto 16 mm cine film and counted using a Geiger tube, or dried onto Whatman no. 1 chromatography paper and counted in butyl PBD–toluene (4 g/l.) scintillator. The release of 7s RNA was achieved by 5 min incubation at 60° in electrophoresis buffer (36 mM NaH_2PO_4, 10 mM EDTA–Na salt, 30 mM Trizma base, 0·2% SDS, final pH 7·4) containing 12% sucrose prior to electrophoresis. Sizes of components on 2·4% gels were determined by co-electrophoresis with *Escherichia coli* *r*RNA ($1{\cdot}1 \times 10^6$ and $0{\cdot}56 \times 10$ daltons) while the MW of the 7s component was determined by comparison with those of the 4s and 5s components (MW's $2{\cdot}5 \times 10^4$ and $3{\cdot}79 \times 10^4$ respectively) [15].

REFERENCES

1. Payne, P. E. and Dyer, T. A. (1972) *Nature, New Biol.* **235,** 145.
2. Pène, J. J., Knight, E. and Darnell, J. E. (1968) *J. Mol. Biol.* **33,** 609.
3. Sy, J. and McCarty, K. S. (1971) *Biochim. Biophys. Acta* **228,** 517.
4. Weinberg, R. A. and Penman, S. (1970) *J. Mol. Biol.* **47,** 169.
5. Leaver, C. J. and Key, J. L. (1970) *J. Mol. Biol.* **49,** 671.
6. Wellauer, P. K. and Dawid, I. B. (1973) *Proc. Nat. Acad. Sci., U.S.* **70,** 2827.
7. Maden, B. E. H. and Robertson, J. S. (1974) *J. Mol. Biol.* **87,** 227.
8. Speirs, J. and Birnstiel, M. (1974) *J. Mol. Biol.* **87,** 237.
9. Choi, Y. C. and Bush, H. (1970) *J. Biol. Chem.* **245,** 1954.
10. Siev, M., Weinberg, R. and Penman, S. (1969) *J. Cell Biol.* **41,** 510.
11. Reeder, R. H. and Brown, D. D. (1970) *J. Mol. Biol.* **51,** 361.
12. Yeoman, M. M. and Davidson, A. W. (1971) *Ann. Botany* **35,** 1085.
13. Leaver, C. J. and Ingle, J. (1971) *Biochem. J.* **123,** 235.
14. Loening, U. E. (1967) *J. Mol. Biol.* **38,** 355.
15. Payne, P. E. and Dyer, T. A. (1971) *Biochem. J.* **124,** 83.