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RIBOSOMES AND RIBOSOMAL PROTEINS OF DRY AND GERMINATING CONIFER SEEDS

JACK A. PITEL* and DON J. DURZAN†‡

* Department of the Environment, Petawawa Forest Experiment Station, Chalk River, Ontario, Canada, K0J 1J0; † Department of the Environment, Policy and Program Development, Environmental Management Service, Ottawa, Canada, K1A 0H3

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Key Word Index—*Pinus banksiana*; Pinaceae; ribosomes; ribosomal proteins; germination; polyacrylamide gel electrophoresis; chloroplasts.

Abstract—The electrophoretic properties of ribosomes and ribosomal proteins of coniferous seeds were determined on polyacrylamide gels. Dry seeds of jack pine (*Pinus banksiana* Lamb.) contained 80S monoribosomes; polysomes were absent. After 48 hr of imbibition the seeds contained monoribosomes and polysomes. The MWs of the ribosomal proteins of the cytoplasm and chloroplasts were 10 to 82 × 10^3 and 9 to 65 × 10^3 respectively. Ribosomal proteins from *Pinus*, *Abies*, and *Pseudotsuga* were electrophoretically similar.

INTRODUCTION

Until recently only a few studies have considered the nitrogen metabolism and protein complement of gymnosperms even though these species are the major land cover of the northern temperate zones and nitrogen is often the main factor limiting tree growth. A better understanding of the value and nature of the proteins synthesized by these widespread species depends on the analytical tools available to the forest researcher. In this context we describe first the electrophoretic properties of monoribosomes, polysomes and their sub-units from jack pine (*Pinus banksiana* Lamb.). These components have been resolvable on polyacrylamide gels. This technique is, in several ways, superior to that of using sucrose density gradients [1, 2–4]. Secondly, the ribosomal proteins of dry and germinating jack pine seeds were examined to determine if, during imbibition, ribosomal proteins changed in numbers and in their properties. So far, limited data are available on the properties of ribosomal proteins from plants [1, 5–7]. Thirdly, the MWs of the basic ribosomal proteins from the cytoplasm and the chloroplasts of jack pine were determined by using SDS (sodium dodecyl sulfate) polyacrylamide gels. Fourthly, the electrophoretic properties of the ribosomal proteins from several commercially important members of the Pinaceae and, for comparative purposes, *Zea mays* were determined to evaluate interspecies variability.

RESULTS AND DISCUSSION

Electrophoresis of ribosomes and sub-units

The ribosome preparation from dry seeds of jack pine gave one band after electrophoresis on 2.2% polyacrylamide gels. The band represented 80 S monoribosomes. Dry seeds of angiosperms [1, 8, 9–12] and of *P. lambertiana* [13] also contain 80 S monoribosomes and are devoid of polysomes. We found no evidence for the presence of bacterial 70 S or chloroplast 70 S ribosomes [14, 15].

The 48-hr imbibed seeds contained 80 S monoribosomes and several heavier components thought to be polysomes. The formation of polysomes is supported by electron microscopy studies of jack pine seeds from the same seed lot [16]. During imbibition, the formation of polysomes arises from pre-existing monoribosomes and preformed *m*RNA present in dry seeds [8]. Formation is rapid [10] and may occur in the absence of *m*RNA transcription [8–10]. In jack pine, the increased polysome population contributes to net protein content of the cytoplasm and is supported by cytochemical observations [16]. The extent of protein synthesis during the early stages of germination in long-lived conifers is of interest in establishing a forecast of seedling and hybrid vigor.

After dialysis of the 80 S ribosomes from dry seeds against 30 μM $MgCl_2$ for 18 hr [13] two sub-unit peaks with sedimentation coefficients of 40 S and 30 S were observed. Three minor bands were detected near the two main bands. Subunits from ribosomes of dry pea seeds also contained minor bands [1]. The hypothesis that the 3 minor peaks represent heterogeneity of the ribosomal sub-units rather than artifacts requires confirmation [2, 3]. In our studies, the electrophoretic technique has proven useful to examine routinely the composition and changes in the ribosome populations of coniferous tissues.

Cytoplasmic ribosomal proteins from dry and germinating seeds

The basic proteins from cytoplasmic ribosomes in dry and germinating jack pine seeds are similar (Fig. 1 (a and b)). Minor quantitative differences were observed. At 48 hr, an additional high MW protein was detected. During the development of angiosperms, changes have been observed in populations of ribosomal proteins. Ten-day-old corn (*Zea mays*) leaves when com-

‡Present address: Institute of Paper Chemistry, Appleton, WI54911, U.S.A.

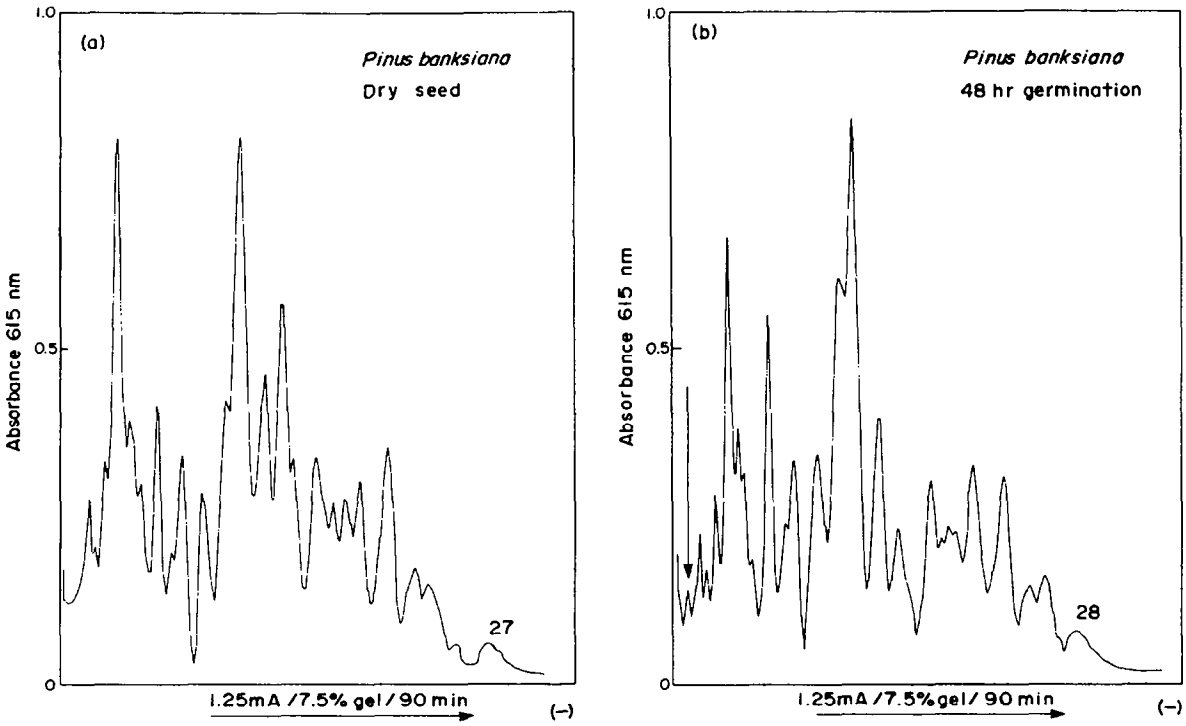


Fig. 1. Comparison of ribosomal proteins from dry and 48-hr imbibed seeds. Ribosomes of jack pine were extracted for the ribosomal proteins with 0.4 M H_2SO_4. Protein (200 μg) was placed on 7.5% urea–acetic acid polyacrylamide gels and electrophoresis proceeded towards the cathode at 20° for 1.5 hr at 1.25 mA/gel. Gels were stained with aniline blue black and scanned at 615 nm. (a) Dry seed preparation, and (b) ribosomal proteins from 48-hr imbibed seeds. The arrow designates the extra protein found at this stage of germination.

pared with two-day-old corn leaves contained four fewer ribosomal proteins [6]. The loss of proteins was associated with a decrease in protein synthesis.

Changes in the ribosomal proteins during germination of cotyledons of mung bean (*Phaseolus aureus*) were reported by Biswas [5]. In 6-hr imbibed cotyledons, levels of two ribosomal proteins decreased by 30% when compared with preparations from 24-hr cotyledons. Changes in the ribosomal proteins have been postulated to permit ribosomal recognition of specific types of *m*RNA. Other variations in the ribosomal proteins during growth and development result in part from specific gene expressions [17]. The significance of minor changes in ribosomal proteins of jack pine during imbibition cannot be answered at present.

Ribosomal proteins from the cytoplasm and chloroplasts

Basic proteins of the chloroplast ribosomes from 14-day-old jack pine seedlings, analyzed on urea–acetic acid gels, are shown in Fig. 2. Although the number of bands is similar to that in the cytoplasmic ribosomal proteins (Fig. 1 (a and b)), the profiles and electrophoretic mobilities of the two sets of proteins are significantly different. Twenty-one bands had different electrophoretic mobilities. The chloroplast ribosomes contained several proteins of higher mobility and had fewer bands of lower mobility than cytoplasmic preparations. Our results are consistent with reported differences between the chloroplast ribosomal proteins and the cytoplasmic ribosomal proteins in spinach (*Spinacea oleracea*) where the lack of homology was established

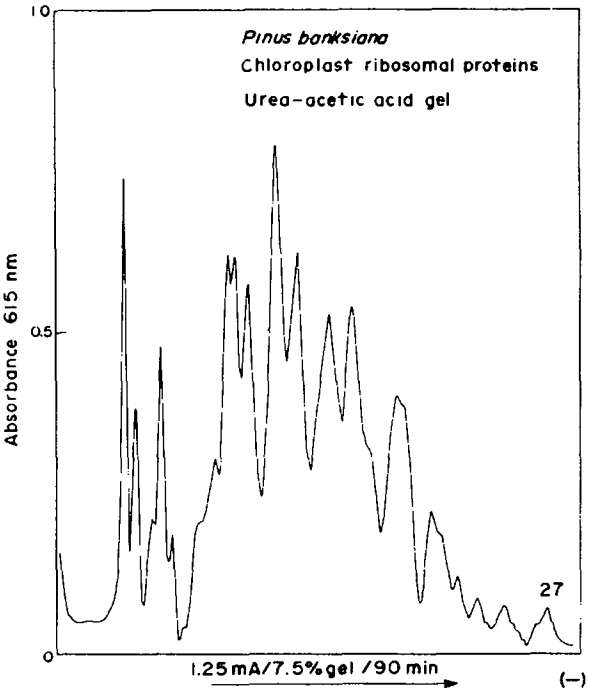


Fig. 2. Basic ribosomal proteins of chloroplast ribosomes. Ribosomal proteins from chloroplasts of 14-day-old jack pine seedlings were analyzed on urea–acetic acid 7.5% polyacrylamide gels. Electrophoresis for 1.5 hr at 1.25 mA/gel proceeded towards the cathode.

Table 1. Estimated MWs of ribosomal proteins from the cytoplasm and chloroplasts of 14-day-old jack pine seedlings. Electrophoresis employed the SDS method of gel preparation

Ribosomal proteins cytoplasm		Ribosomal proteins cytoplasts	
Protein	MW ($\times 10^3$)*	Protein	MW ($\times 10^3$)*
1	82	1	65
2	73	2	58
3	64	3	54
4	59	4	53
5	54	5	48
6	45	6	42
7	37	7	35
8	36	8	34
9	34	9	32
10	30	10	27
11	28	11	25
12	23	12	20
13	20	13	18
14	17	14	17
15	16	15	15
16	15	16	13
17	13.5	17	12.5
18	13	18	12
19	12	19	11
20	11	20	10
21	10.5	21	9
22	10		
W_{TN}†	31.8	W_{TN}†	29.0

* Mean of duplicate determinations.

† W_{TN} is the number average MW (total MWs of protein divided by the total number of proteins).

by immunological tests and by two-dimensional electrophoresis [18, 19].

Jack pine chloroplasts from 14-day-old seedlings were judged by light microscopy to be free from nuclear and cytoplasmic fragments. Electrophoresis of RNA from chloroplast ribosomes did not reveal cytoplasmic 25S and 18S RNA components.

The ribosomal proteins of the cytoplasm and chloroplasts of jack pine contained different complements of acidic proteins. Acidic proteins of cytoplasmic ribosomes were resolved into 3 faint bands. Chloroplast ribosomes gave at least 9 bands. In spinach [18] the acidic ribosomal proteins from chloroplasts separated into 11–12 bands compared with only a few faint bands from the cytoplasmic ribosomes. In *Euglena gracilis* [20], 7 acidic proteins were observed in the chloroplast preparation and only 2 lightly staining components in the cytoplasmic preparation. The acidic ribosomal proteins from cytoplasmic ribosomes of jack pine are similar to those of most eukaryotes, which contain only a few faint acidic ribosomal proteins [20].

The MWs of the basic ribosomal proteins (Table 1) calculated on SDS gels [21], range from 10 to 82 $\times 10^3$ (cytoplasmic ribosomal proteins) and from 9 to 65 $\times 10^3$ (choloroplast ribosomal proteins). Five proteins between the two extracts had similar MWs. The chloroplast preparation had fewer proteins and had a lower W_{TN} (number average MW) than did the cytoplasmic ribosomes. Similar results were observed in *E. gracilis* [20]. The cytoplasmic ribosomes had more proteins than the chloroplast ribosomes. Also ribosomal proteins of the cytoplasm had a higher W_{TN} than the ribosomal proteins from chloroplasts. In pea seeds [1] the ribosomal proteins of the cytoplasm had a MW range of 10 to 78 $\times 10^3$ with a W_{TN} (60S sub-unit) of 34 and a W_{TN} (40S sub-unit) of 26. Most proteins varied from 10 to 35 $\times 10^3$. The MWs of ribosomal proteins from jack pine are consistent with those in reports for other plants.

Ribosomal proteins of the Pinaceae

Ribosomal proteins have been reported to vary widely in composition among genetically distant species. However, proteins are similar over a wide range of species within the same class [22, 23].

Ribosomal proteins of the Pinaceae followed this general rule (Fig. 3(a–f)). Minor quantitative and qualitative differences were observed in electrophoretic profiles. Differences were more apparent among the higher MW proteins. The number of proteins is very similar, i.e. 24–27 fractions. Arrows on the Figs indicate the absence of a protein when compared with the ribosomal profile for jack pine (Fig. 1a). In *Abies balsamea* three proteins were absent.

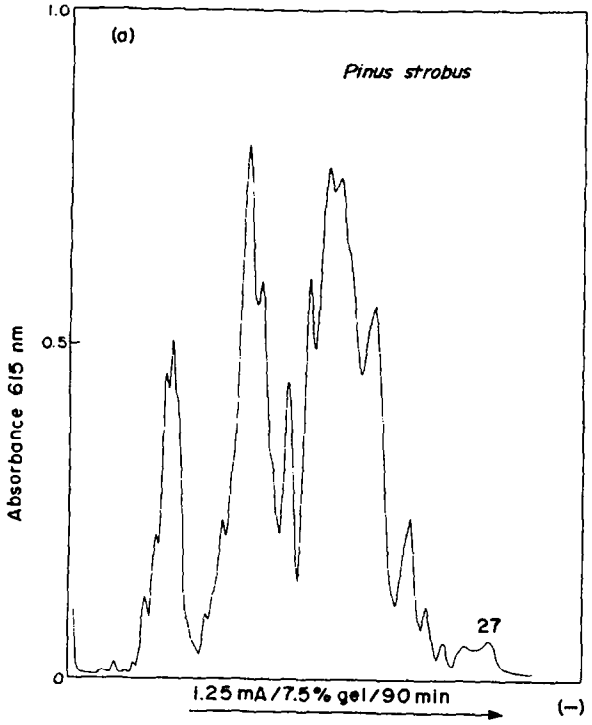
For *P. strobus* the ribosomal proteins appeared identical in electrophoretic mobility to those from *P. banksiana*. However, co-electrophoresis of the two sets of proteins in the same gel should be done to obtain a more positive conclusion regarding identical mobility. Comparison of ribosomal proteins from *Zea mays* (Fig. 3g) with those from conifer seeds reveal significant differences. These included a larger number of proteins in *Zea mays* and also numerous quantitative differences.

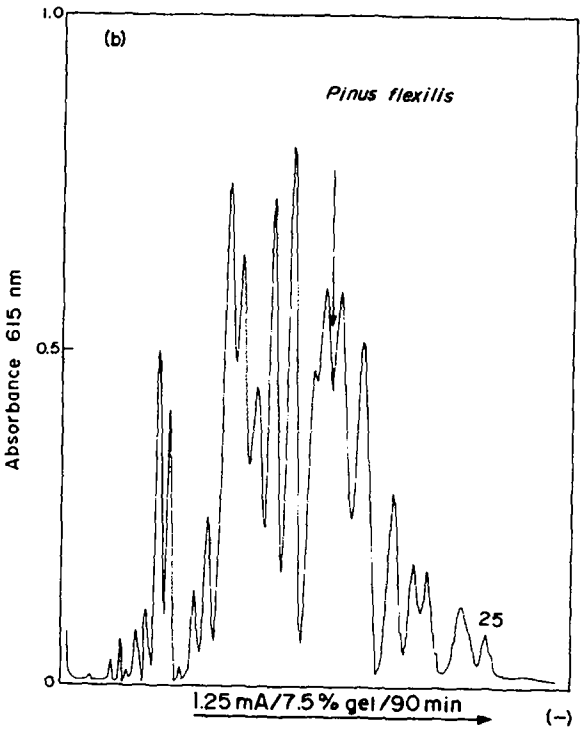
Strong similarities in the cytoplasmic ribosomal proteins of closely related angiosperms exist. Between members of the same family, such as kidney bean and lentil (Papilionaceae), the electrophoretic mobilities of the ribosomal proteins after two-dimensional electrophoresis are believed identical, whereas numerous differences were found between members of two families such as maize (Gramineae) and lentil [22]. Our results with conifers extend these observations and are consistent with studies with other plants and animals that show a high degree of homology in the ribosomal proteins for closely related species.

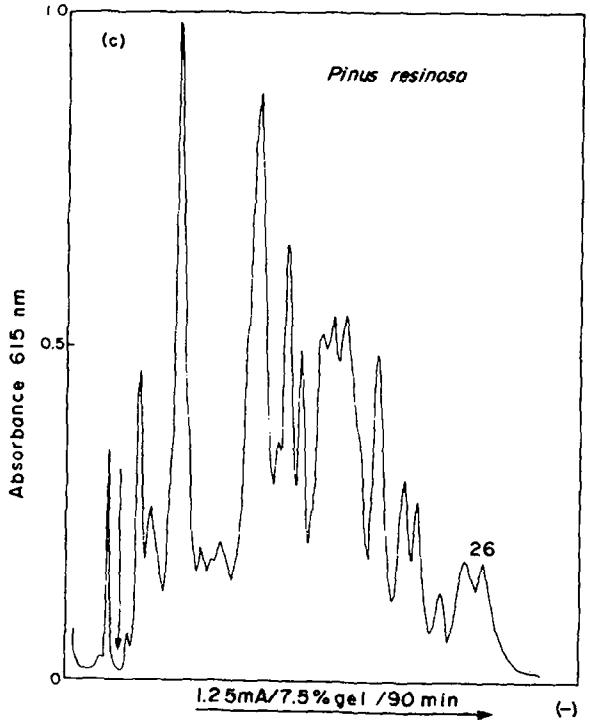
EXPERIMENTAL

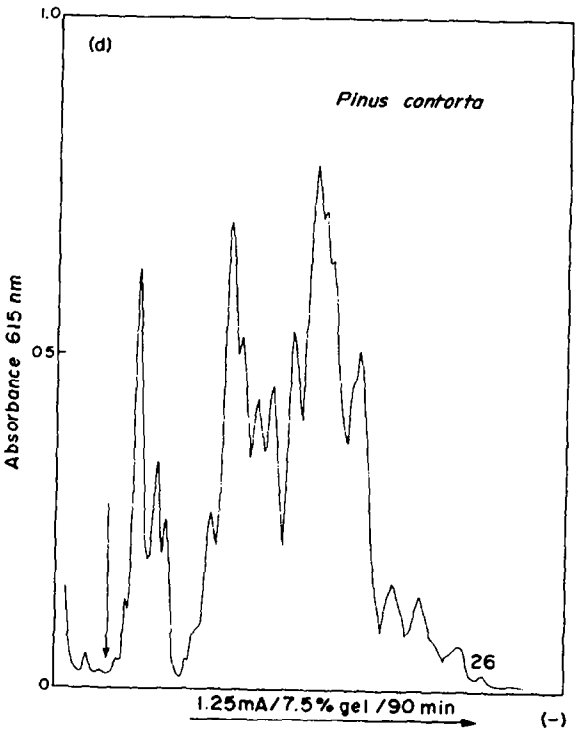
Materials and growth conditions. The following seed lots of not less than 95% germination at day 4 were obtained from Petawawa Forest Experiment Station, Chalk River, Ontario Canada: jack pine (*Pinus banksiana* Lamb; Petawawa Plains, Ontario), white pine (*Pinus strobus* L.; Petawawa Plains, Ontario), lodgepole pine (*Pinus contorta* Dougl.; Whitecourt, Alberta), red pine (*Pinus resinosa* Ait.; Temagami, Ontario), limber pine (*Pinus flexilis* James; Montana), balsam fir (*Abies balsamea* [L.] Mill.; Petawawa Plains, Ontario) and Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco; Pinetan, British Columbia). Corn seeds (*Zea mays*) with germination greater than 96% were purchased locally. Conifer seeds were surface-sterilized with 5% Ca hypochlorite followed by washings with H_2O and then incubated at 20° under continuous light. Light energy supplied was 5.8, 4.9, and 2.4 $\mu W/cm^2$ in the blue, red and far-red, respectively. Corn seeds were geminated in the dark at 20° for 4 days, after which entire shoots were excised, surface-sterilized and extracted for ribosomes.

Preparation of cytoplasmic ribosomes. Dry seeds or seedlings were homogenized at 4° in a Waring blendor at 60 V for 2 min with 4 vol. of buffering medium composed of 50 mM Tris–HCl


(a)
Pinus strobus
1.0
0.5
0
Absorbance 615 nm
27
1.25 mA/7.5% gel/90 min
(−)


(b)
Pinus flexilis
1.0
0.5
0
Absorbance 615 nm
25
1.25 mA/7.5% gel/90 min
(−)


(c)
Pinus resinosa
1.0
0.5
0
Absorbance 615 nm
26
1.25mA/7.5% gel /90 min
(−)


(d)
Pinus contorta
1.0
0.5
0
Absorbance 615 nm
26
1.25mA/7.5% gel /90 min
(−)

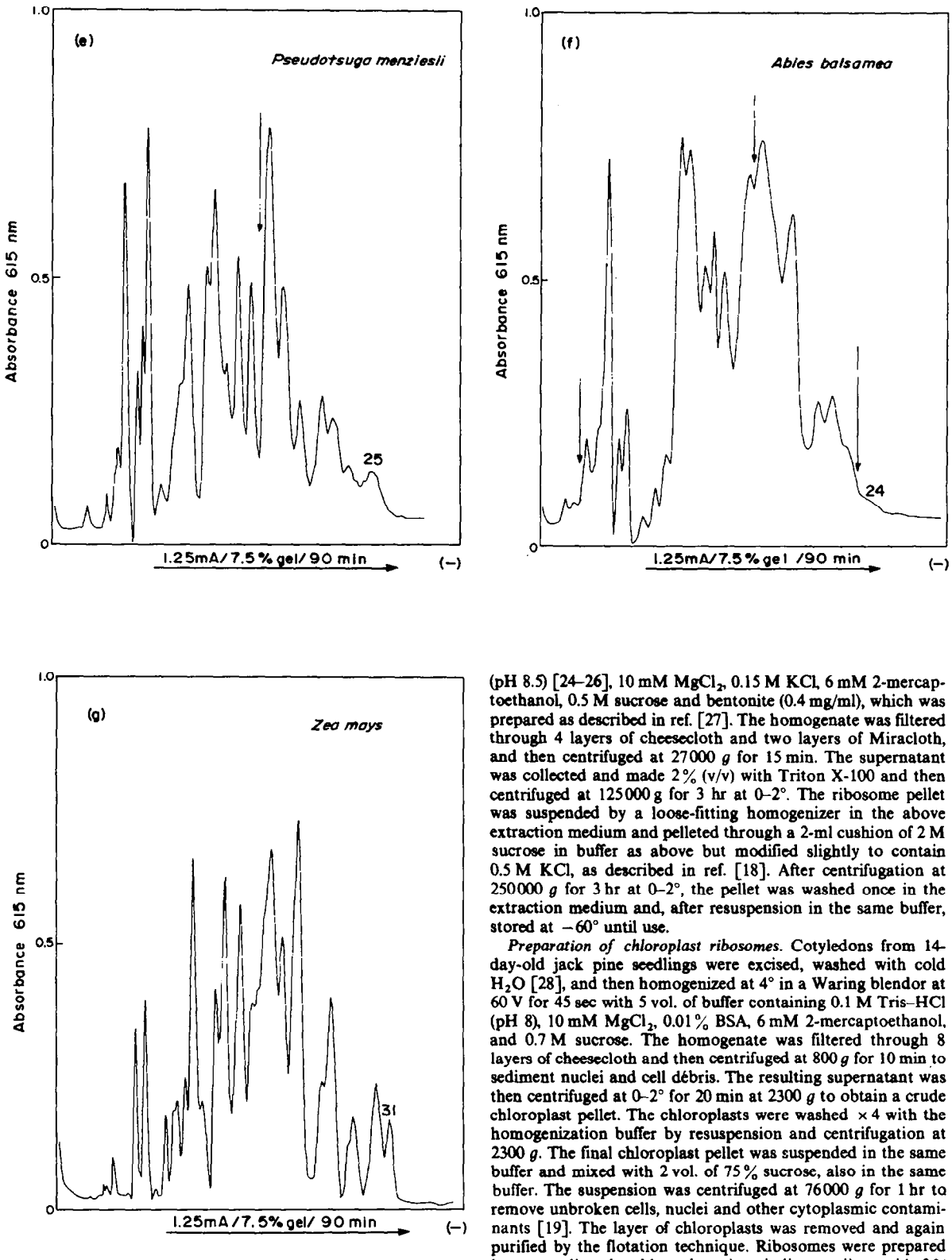


Fig. 3. Comparison of ribosomal proteins from several members of the Pinaceae. Basic ribosomal proteins from species of pine, fir and corn on urea–acetic acid polyacrylamide gels. The species are (a) *Pinus strobus*, (b) *Pinus flexilis*, (c) *Pinus resinosa*, (d) *Pinus contorta*,, (e) *Pseudotsuga menziesii* (Mirb.) Franco, (f) *Abies balsamea* (L.) Mill. and (g) *Zea mays*.

(pH 8.5) [24–26], 10 mM $MgCl_2$, 0.15 M KCl, 6 mM 2-mercaptoethanol, 0.5 M sucrose and bentonite (0.4 mg/ml), which was prepared as described in ref. [27]. The homogenate was filtered through 4 layers of cheesecloth and two layers of Miracloth, and then centrifuged at 27000 *g* for 15 min. The supernatant was collected and made 2% (v/v) with Triton X-100 and then centrifuged at 125000 g for 3 hr at 0–2°. The ribosome pellet was suspended by a loose-fitting homogenizer in the above extraction medium and pelleted through a 2-ml cushion of 2 M sucrose in buffer as above but modified slightly to contain 0.5 M KCl, as described in ref. [18]. After centrifugation at 250000 *g* for 3 hr at 0–2°, the pellet was washed once in the extraction medium and, after resuspension in the same buffer, stored at −60° until use.

Preparation of chloroplast ribosomes. Cotyledons from 14-day-old jack pine seedlings were excised, washed with cold H_2O [28], and then homogenized at 4° in a Waring blendor at 60 V for 45 sec with 5 vol. of buffer containing 0.1 M Tris–HCl (pH 8), 10 mM $MgCl_2$, 0.01% BSA, 6 mM 2-mercaptoethanol, and 0.7 M sucrose. The homogenate was filtered through 8 layers of cheesecloth and then centrifuged at 800 *g* for 10 min to sediment nuclei and cell débris. The resulting supernatant was then centrifuged at 0–2° for 20 min at 2300 *g* to obtain a crude chloroplast pellet. The chloroplasts were washed × 4 with the homogenization buffer by resuspension and centrifugation at 2300 *g*. The final chloroplast pellet was suspended in the same buffer and mixed with 2 vol. of 75% sucrose, also in the same buffer. The suspension was centrifuged at 76000 *g* for 1 hr to remove unbroken cells, nuclei and other cytoplasmic contaminants [19]. The layer of chloroplasts was removed and again purified by the flotation technique. Ribosomes were prepared by suspending the chloroplasts in grinding medium with 2% Triton X-100. After homogenization with a loosely fitted homogenizer at 4°, the homogenate was centrifuged at 27000 *g* for 30 min. The pellet was discarded and the supernatant centrifuged at 125000 *g* for 3 hr. The ribosome pellet was suspended in grinding medium without sucrose and layered over a 2 ml-cushion of 40% sucrose in buffer containing 2% Triton

X-100 before centrifugation for 6 hr at 0–2° at 250000 *g*. The chloroplast ribosomes were repelleted through dense sucrose and then stored at −60°.

Isolation of ribosomal proteins. Ribosomes from dry seeds, 48-hr imbibed seeds, 14-day-old cotyledons and 4-day-old corn shoots were extracted with 0.4 M H_2SO_4 [29] at 4°. Insoluble RNA was removed by centrifugation at 27000 *g* for 15 min. After re-extraction of the ppt., the supernatants were combined and 4 vol. of cold EtOH were added to ppt. the protein. Precipitation continued for 18 hr at −20°. Extractions with 66% HOAc [30] and also a mixture of 4 M LiCl and 8 M urea were used. In the latter method the ribosomes were suspended in 1 mM Tris–HCl (pH 7.8) buffer and mixed with an equal vol. of 8 M urea–4 M LiCl. After 18 hr precipitation the RNA was removed by centrifugation (20000 *g*) and the supernatant was dialyzed for 48 hr against 1 mM Tris–HCl (pH 7.8) buffer in 6 M urea and 5 mM mercaptoethanol. Proteins were re-precipitated with EtOH as above.

Electrophoresis of ribosomal proteins. The urea–HOAc method was used for electrophoresis of the basic ribosomal proteins [31]. In all cases 7.5% polyacrylamide gels were used. The gels were overlain with 200 μg of protein in 6 M urea–0.9 N HOAc containing 5% sucrose and 6 mM 2-mercaptoethanol. After electrophoresis towards the cathode for 1.5 hr (1.25 mA/gel), the gels were removed from the tubes and stained with 0.5% aniline blue black in 10% HOAc. Gels were destained by diffusion with numerous washings with 10% HOAc and scanned at 615 nm. MWs were determined by the SDS method of ref. [21]. Ribosomal proteins, pptd and washed with EtOH, were dissolved in 10 mM Pi buffer, pH 7, containing 1% SDS, 1% 2-mercaptoethanol and 10% sucrose, and then placed at 37° for 2 hr. The gel soln contained 10% acrylamide, 0.27% bisacrylamide, 0.1% SDS, 0.15% TEMED and 0.075% NH_4 persulfate in 10 mM NaPi buffer (pH 7). Samples with 200 μg of ribosomal protein were applied to gels and electrophoresis proceeded towards the anode at 5 mA/gel for 2 hr at room temp. with bromophenol blue as a marker. Protein standards used for MW determinations were: BSA, 68000; ovalbumin, 46500; chymotrypsinogen A, 25700; and cytochrome *c*, 13500. Gels were removed and stained with 0.1% Coomassie brilliant blue in 45% EtOH–10% HOAc for 15 min at 65°. Gels were destained by washings at 65° with a soln containing 25% EtOH–10% HOAc. The MWs of the ribosomal proteins were calculated after plotting the relative distance of migration to tracking dye of the standards against the log of their MWs. Electrophoresis of the acidic ribosomal proteins was performed on discontinuous gels as described in ref. [32]. Analysis was on pH 8.5, 6 M urea, 7.5% polyacrylamide gels. Protein (200 μg) was applied to the gel and electrophoresis proceeded towards the anode at 5 mA/gel until the bromophenol blue tracking dye reached the end of the gel. Gel staining was with Coomassie brilliant blue as already described.

Electrophoresis of ribosomes and ribosomal sub-units. Ribosomes were analyzed on 2.2% polyacrylamide gels [33] using recrystallized acrylamide and *N,N'*-methylene-bisacrylamide. Electrophoresis buffer and conditions were essentially those described in ref. [4]. Direction of migration was towards the anode at 5 mA/gel for 2 hr. Gels were removed and, after several washes with 7% HOAc, were scanned unstained at 260 nm. Sub-units, obtained by dialyzing a ribosome suspension from dry jack pine seeds against 0.01 M Tris–HCl (pH 8) buffer containing 0·03 mM $MgCl_2$ for 18 hr, were fractionated on 2.2% polyacrylamide gels using the electrophoresis buffer containing Na PPi [1]. *Protein content* was determined with commercial BSA as a standard [34].

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