## SHORT NOTE

# Prosomes Exist in Plant Cells Too

ANETTE KREMP,[1] MARTIN SCHLIEPHACKE,[1] ULRICH KULL[1] and HANS-PETER SCHMID[2,*]

*Biologisches Institut der Universität Stuttgart, Abteilung für [1]Pflanzenphysiologie und [2]Zoologie, D-7000 Stuttgart 60, FRG*

A 19S particle was purified from tobacco (*Nicotiana rustica*) leaf cells. Its density was determined as 1 296 g/cm$^3$ in $Cs_2SO_4$-DMSO gradients, indicating the presence of RNA and protein. Polyacrylamide gel electrophoresis (PAGE) revealed eight distinct proteins in the range of 20–30 kD and RNA in the range of 70–80 nucleotides. Electron microscopic examination showed the same raspberry-shaped structure with a central depression as described for prosomes. We conclude that tobacco 19S particles represent small cytoplasmic complexes, possessing biochemical and structural characteristics similar to the hitherto known prosomes of animal cells. 

Prosomes, novel and ubiquitous small ribonucleoprotein complexes (19S ScRNPs) consist of a specific set of proteins associated with small RNA. Their RNA content seems to be cell type-specific, since prosomes of erythroblasts, *Drosophila* and HeLa cells revealed different RNA patterns in one- (1D) or two-dimensional (2D) gel electrophoresis [1–3].

Some of the proteins are similar to the small heat shock proteins as reported for the prosomes of *Drosophila* cells [2, 4]. Both heat shock proteins and prosomal proteins are highly conserved during evolution [3, 5].

Furthermore the prosomes resist (unfixed) the rather strong detergent sodium *N*-lauroylsarcosinate as well as cesium sulfate–DMSO centrifugation (density 1.3 g/cm$^3$) [1].

Prosomes were recently found associated with repressed free mRNP complexes in various animal and human cells [1]. Earlier investigations suggested that prosomes play an important role as control factors of cytoplasmatic gene expression. They tend to associate strongly with viral mRNA and inhibit their translation in a cell-free system [6].

We now present evidence that prosomes also exist in plant cells.

## *Materials and Methods*

*Tobacco cell fractionation and isolation of 19S particles.* Tobacco (*Nicotiana rustica*) leaves were homogenized in 200 mM Tris-HCl (pH 8.5), 100 mM KCl, 35 mM $MgCl_2$, 25 mM EGTA, 7 mM 2-mercaptoethanol, 200 mM sucrose and filtered through Miracloth. Clarification was followed by centrifugation (WKF rotor 6×100, 40 min, 10 000 rpm, 4°C). Differential ultracentrifugation yielded preparations of polyribosomes and post-ribosomal particles [1].

Free cytoplasmic ribonucleoprotein complexes were fractionated by sedimentation of the resuspended post-ribosomal pellets in 10–25% w/w sucrose gradients in 20 mM Tris-HCl (pH 7.4), 100 mM

* To whom offprint requests should be sent. Address: Biologisches Institut, Universität Stuttgart, Ulmer Strasse 227, D-7000 Stuttgart 60, FRG.

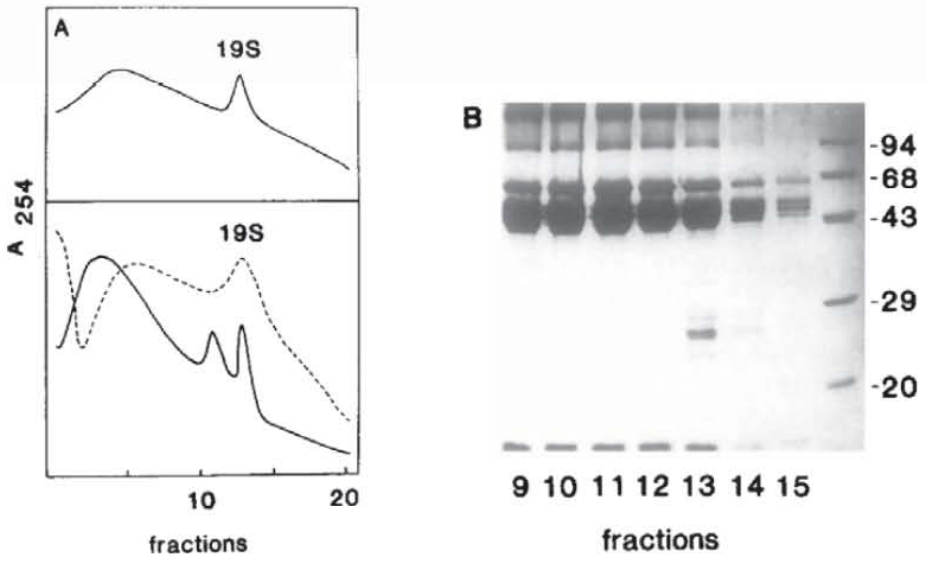


*Fig. 1.* Purification and analysis of tobacco 19S particles. (*A*) Sucrose gradient analysis of 19S particles. Fractionated free cytoplasmatic RNP complexes in the range of 10S–30S were pooled, concentrated by high-speed centrifugation (Beckman rotor Ti 60, 18 h, 48 000 rpm, 4°C), resuspended in sarcosyl-buffer and subjected to centrifugation in a 10–50 % w/w sucrose gradient containing the same buffer ——, $A_{254}$ absorbancy profile (range 1 $A_{254}$) of the fractionated sarcosyl sucrose gradients of (*top*) mouse; (*bottom*) tobacco. – – –, $A_{254}$ absorbancy profile (range 0.4 $A_{254}$ of further purified 19S particles of tobacco. (*B*) Laemmli SDS–PAGE of proteins visualized by Coomassie Blue stain. After purification of tobacco 19S particles by 10–50 % w/w sucrose gradients containing sodium *N*-lauroyl-sarcosinate, the protein pattern of fractions 9–15 was analysed. Marker proteins were phosphorylase b (94 kD), bovine serum albumin (68 kD), ovalbumin (43 kD), carbonic anhydrase (29 kD) and soybean trypsin inhibitor (20 kD).

KCl, 3 mM $MgCl_2$, 7 mM 2-mercaptoethanol (Beckman rotor SW 27, 20 h, 18 000 rpm, 4°C). Particles sedimenting between 30S and 10S were pooled and concentrated by high-speed centrifugation (Beckman rotor Ti 60, 18 h, 48 000 rpm, 4°C). The pellets were resuspended in sarcosyl-buffer (20 mM Tris–HCl (pH 7.4), 7 mM 2-mercaptoethanol, 1 % w/w sodium *N*-lauroyl-sarcosinate) and sedimented in 10–50 % w/w sucrose gradients containing the same buffer (Beckman rotor SW 40, 25 h, 38 000 rpm, 4°C). Prosomes of mouse erythroblasts were sedimented in the same rotor.

For further purification pooled 19S fractions were sedimented again through 10–50 % w/w sucrose gradients containing 1 % w/w sodium *N*-lauroylsarcosinate. Conditions of centrifugation were the same as above.

*$Cs_2SO_4$–DMSO density centrifugation.* Buoyant density of tobacco 19S particles was determined in a MSE Centriscan 75 (MSE six-place analytical rotor, 67 h, 40 000 rpm, 20°C) in 20 mM Tris-HC1 (pH 7.4), 10 mM KCl, 0.01 % v/v Triton X-100, 15 % v/v DMSO containing 25 % w/w $Cs_2SO_4$. Calculation was carried out according to Vinograd [7].

*Gel electrophoresis of proteins and RNA.* Electrophoresis of proteins in 1D 12.5 % polyacrylamide gels was performed according to Laemmli [8]. MW markers were phosphorylase *b* (94 kD), bovine serum albumin (68 kD), ovalbumin (43 kD), carbonic anhydrase (29 kD) and soybean trypsin inhibitor (20 kD). The proteins were visualized by Coomassie Blue stain.

RNA labelling and electrophoresis was performed as described by Schmid et al. [1].

*Electron microscopy.* A drop of the appropriate sucrose gradient fractions (cf fig. 1) was deposited onto Formvar-coated copper grids. Excess liquid was removed with filter paper and the grids were stained with 0.1 % w/v uranylacetate in double-distilled water and dried in air. Micrographs were taken with a Zeiss EM 10 % electron microscope at 80 kV. The diameter of the particles was measured on prints enlarged photographically to ×258 000.

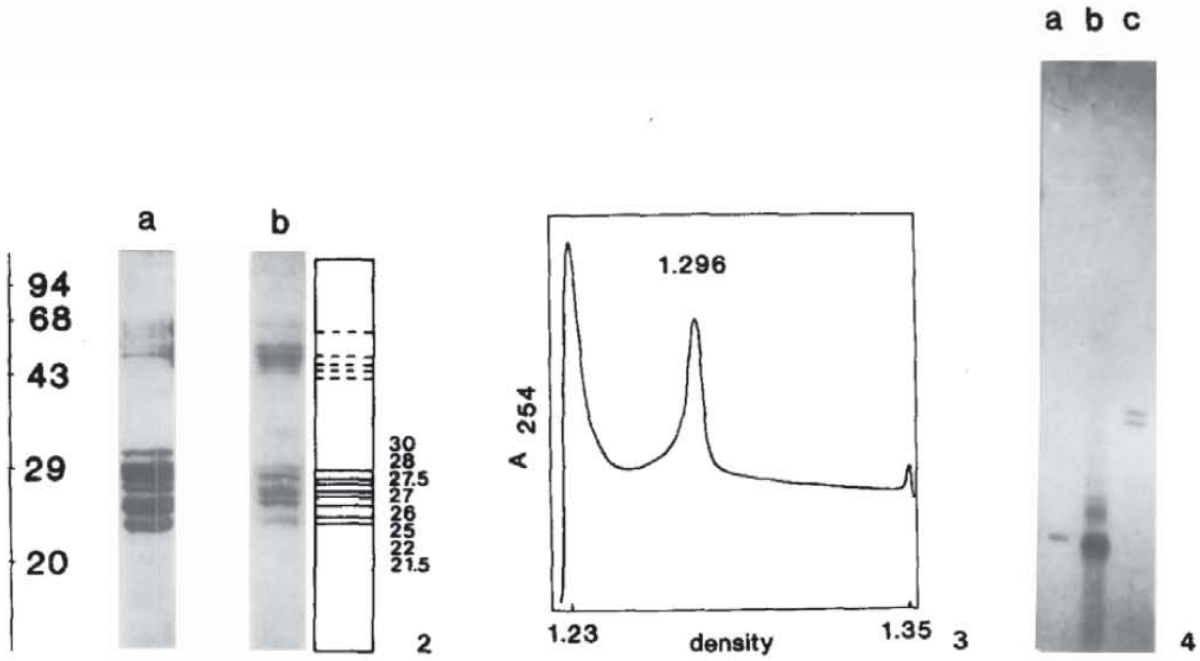


*Fig. 2.* Laemmli SDS–PAGE of further purified tobacco 19S particles. Lanes: Protein patterns of *a*, mouse prosomes purified through sarcosyl sucrose gradients; *b*, 19S peak fraction of the second sucrose gradient containing sodium *N*-lauroylsarcosinate. Sizes are shown in kD and proteins are visualized by Coomassie Blue stain.

*Fig. 3.* Buoyant density ($Cs_2SO_4$) of tobacco 19S particles. The buoyant density was determined in a MSE Centriscan 75 (MSE six-place analytical rotor, 67 h, 40000 rpm, 20°C) and calculation was carried out according to Vinograd [7].

*Fig. 4.* Urea PAGE of RNA labelled at the 3′ end with $^{32}$pcp. Lanes: *a*, Phe-tRNA (Boehringer); *b*, RNA composition of tobacco 19S particles purified through sarcosyl sucrose gradients; *c*, 5S-RNA (Boehringer).

## *Results and Discussion*

Post-ribosomal supernatants of tobacco (*Nicotiana rustica*) leaf cells containing particles in the range of 30S–10S were sedimented through sucrose gradients with 1% w/w sodium *N*-lauroylsarcosinate. Two fractions of particles obviously resisted these drastic conditions. One fraction settled in the range of 19S at the same position as purified prosomes of mouse erythroblasts, which sedimented as markers in a parallel tube in the same rotor (fig. 1*A*).

Fractions 9–15 were collected and analysed by SDS–PAGE. Fig. 1*B* demonstrates that fractions 13 and 14 (19S) contain proteins in the MW range of 20–30 kD, which is similar to that of mouse prosomes.

However, in the range of 50–65 kD there are prominent proteins which are also very abundant in all other fractions. To avoid contamination by other closely sedimenting particles, fractions 13 and 14 were pooled and centrifuged again through sucrose gradients containing 1% w/w sodium *N*-lauroylsarcosinate.

Further analysis of 19S particles (cf fig. 1*A*) by SDS–PAGE revealed now less intensive protein bands in the range of 50–65 kD (fig. 2*b*). They are probably not constituents of the 19S particle.

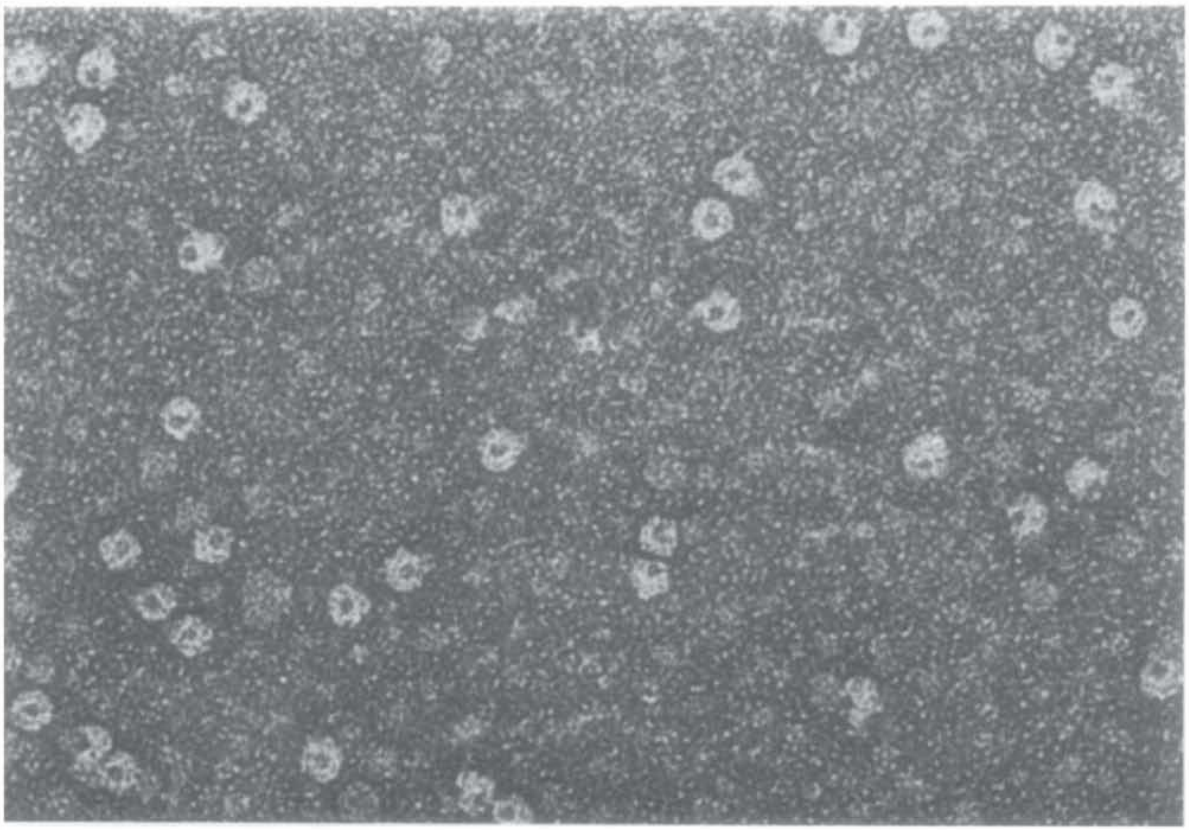

*Fig. 5.* Electron micrograph of tobacco 19S particles. The electron micrograph of purified tobacco 19S particles from sucrose gradient fractions shows round raspberry-shaped particles with a central depression. Grids were stained with 0.1% w/v uranyl acetate. ×258 000.

Interestingly, the protein pattern of mouse prosomes purified through detergent-containing sucrose gradients also reveals some faint bands in the same molecular range (fig. 2*a*), which were identified as cytoskeleton proteins [9].

Eight distinct protein bands are visible with apparent MWs of 21.5 kD, 22 kD, 25 kD, 26 kD, 27 kD, 27.5 kD, 28 kD and 30 kD. Some bands coincide directly with proteins of mouse prosomes.

$Cs_2SO_4$–DMSO density centrifugation revealed 19S particles with a density of 1.296 g/cm$^3$ (fig. 3). This is characteristic for prosomes (density 1.3–1.31 g/cm$^3$) [1, 4], corresponding to a RNA : protein ratio of 1 : 8, while pure proteins sedimented at a buoyant density of 1.18–1.2 g/cm$^3$ [10]. 1D RNA gel electrophoresis also confirmed the presence of small RNAs. Fig. 4 shows that the 19S fraction contains RNA with a size of 70–80 nucleotides which migrates in the same range as RNA of mouse prosomes [1].

Finally the 19S fraction of sucrose gradients was examined by electron microscopy (fig. 5). The micrograph reveals a quite homogeneous population of round raspberry-shaped particles with a diameter of 16 nm. The characteristic structural feature of these particles, which show the same highly organized morphology as mouse prosomes, is their central depression.

We have shown here that 19S particles (prosomes) of tobacco leaf cells represent small cytoplasmatic complexes, having biochemical and structural characteristics similar to those of the hitherto known prosomes of animal cells.

They consist of a specific set of proteins. Some of them are similar to the so-called small heat shock proteins [2]. Schuldt & Kloetzel [4] demonstrated that 19S particles of *Drosophila* cells contain hsp 23 at normal growth temperature. The small heat shock proteins are also abundant in plant cells [5] and it would be of interest to know if these proteins correlate with the proteins of plant prosomes.

We are reduced to allegations based on structure and occurrence of prosomes in relation to species, cell type, cell compartment and their influence of in vitro translation, because their function in the cell is still unknown.

Prosomes were found associated with repressed mRNPs in the cytoplasm of duck and mouse erythroblasts and HeLa cells [1, 3] and in vitro they suppress the translation of mRNA of adenovirus-infected HeLa cells, while they have no influence on the protein synthesis of mRNA isolated from uninfected HeLa cells [6, 11].

Furthermore, analogous to ribosomes of wheat germ system, which are able to translate animal mRNAs, prosomes of animal cells influence the translation of plant virus (tobacco mosaic virus) mRNA in a reticulocyte system [11]. This may be explained by the highly conserved structure of prosomes and points to a system which is fundamental in cellular events, suggesting that these particles play an important role in regulation of eukaryotic cells.

We are most grateful to K. Koehler for critical reading of the manuscript, G. Adam for helpful discussions, H. Ungibauer-Kremp for density centrifugation, D. F. Huelser (Department of Biophysics) for providing the electron microscope and A. Horsch for taking electron micrographs.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Schm 620/1–1).

## *References*

1. Schmid, H P, Akhayat, O, Martins de Sa, C, Puvion, F, Koehler, K & Scherrer, K, EMBO j 3 (1984) 29.
2. Arrigo, A P, Darlix, J L, Khandjian, E W, Simon, M & Spahr, P F, EMBO j 4 (1985) 399.
3. Martins de Sa, C, Grossi de Sa, M F, Akhayat, O, Broders, F, Scherrer, K, Horsch, A & Schmid, H P, J mol biol 187 (1986) 479.
4. Schuldt, C & Kloetzel, P M, Dev biol 110 (1985) 65.
5. Nover, L, Hellmund, D, Neumann, D, Scharf, K D & Serfling, E, Biol Zbl 103 (1984) 375.
6. Horsch, A, Spindler, E, Martins de Sa, C, Koehler, K & Schmid, H P, Proc natl acad sci US (1986). Submitted for publication.
7. Vinograd, J, Methods in enzymol 6 (1963) 854.
8. Laemmli, U K, Nature 227 (1970) 680.
9. Menrad, A. Personal communication.
10. Gaedigk, R, Oehler, S, Koehler, K & Setyono, B, FEBS lett 179 (1985) 201.
11. Horsch, A, Koehler, K & Schmid, H P, Z Naturforsch 40 (1985) 449.

Received April 11, 1986
Revised version received June 6, 1986