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Some Evidence for the Occurrence of Ribonucleic Acid in the Lutoid Fraction (Lysosomal Compartment) from *Hevea brasiliensis* Latex

By BERNARD MARIN* and P. TROUSLOT
Laboratoire de Physiologie Végétale, Centre O.R.S.T.O.M. d'Adiopodoumé, Abidjan, Côte-d'Ivoire
and S. PUJARNISCLE
Laboratoire de Physiologie Végétale, Université du Dahomey, Porto-Novo, Dahomey

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Some results are reported for the presence of some RNA species in the lutoid fraction from *Hevea brasiliensis* latex. The origin of this RNA is discussed.

The latex of *Hevea brasiliensis* MULL-ARG is a complex suspension of polyterpenic particles and organelles including Frey-Wyssling particles and lutoids in a fluid so-called 'serum.' Cytological and biochemical data have indicated that the latex is the cytoplasm of the highly differentiated latex-containing cell (Cockbain & Southorn, 1962). Usually in latex neither nuclei nor mitochondria can be detected by using biochemical techniques (S. Pujarniscle, unpublished work). This is confirmed by electron-microscopic observations (Dickenson, 1965). The only organelles known to be present in latex are Frey-Wyssling particles and lutoids.

RNA has been found in latex (McMullen, 1962; Coupé & d'Auzac, 1972) and has been shown to be associated with the serum fraction (Tupy, 1969), but, so far, polymerized RNA has never been detected in the lutoid fraction. We report here the first evidence of polynucleotides in the lutoids.

The particulate fraction is easily separated from the remainder of the latex by differential centrifugation. One or two washings are sufficient to eliminate some obvious serum contamination (B. Marin, unpublished work). Lutoids and Frey-Wyssling particles can be separated by sucrose-density-gradient centrifugation. Each organelle can be characterized by specific enzyme activity. Acid phosphatase is a marker enzyme for lutoids and polyphenol oxidase for Frey-Wyssling particles (Pujarniscle, 1969).

Fig. 1 shows results obtained from a series of isopycnic sucrose-gradient centrifugations of crude latex and of progressively more purified lutoid fractions. Centrifugation of crude latex clearly resolves the acid phosphatase and polyphenol oxidase activities (Fig. 1*a*). RNA is mainly present in the serum fraction, but a small amount of RNA is also associated with the lutoid fraction, as shown by comparison with the distribution of acid phosphatase activity. About 15–30% of the latex RNA is found in this fraction. Sucrose-gradient centrifugation of a crude lutoid fraction confirmed the presence of RNA in this fraction (Fig. 1*b*). Acid phosphatase and polyphenol oxidase are very well separated and the distribution of RNA parallels very closely that of acid phosphatase activity. Further purification of the lutoid fraction by sucrose-density-gradient centrifugation does not alter the association of RNA with acid phosphatase activity (Fig. 1*c*). At this purification step, no association of RNA with polyphenol oxidase activity is observed. The RNA present in the lutoid fraction is strictly associated with acid phosphatase activity. The ratio of RNA content to acid phosphatase activity/mg of protein remained unchanged during the two successive purifications in sucrose gradients. The average RNA content of such purified lutoid fractions was $6.2 \pm 1.2 \mu g$/mg of protein.

RNA cannot be washed away from the lutoid fraction by repeated EDTA treatments (up to 50mM). Thus non-specific adsorption of serum ribosomes or RNA on lutoid membrane appears to be ruled out. No cytochrome *P*-450 is present in the lutoid fraction (F. Moreau, unpublished work). As this component is present in microsomal membranes, contamination by endoplasmic reticulum structures is eliminated.

Drastic treatment is necessary to release RNA from the lutoid fraction. For example, 80% of the RNA content of the lutoid fraction is released after a 10min treatment with Triton X-100, which solubilizes the lutoid membrane, as shown by the solubilization of the marker enzyme (B. Marin, unpublished work).

We conclude from these results that some RNA found in the lutoid fraction can be present in the lutoid organelle. The results raise the question of the nature of this RNA species. Its base composition determined by the method of Yoshida & Shibata (1969) is rich in G+C nucleotides (A, 21.7%; C, 31.2%; G, 28.9%; U, 18.2%). This distribution is similar to an rRNA base composition. Whether this RNA is a new discrete RNA species or is related to known latex RNA sequences is not yet resolved and

* Present address: Laboratoire de Biologie Moleculaire Végétale, Université de Paris-Sud, Centre d'Orsay, 91.405 Orsay, France.

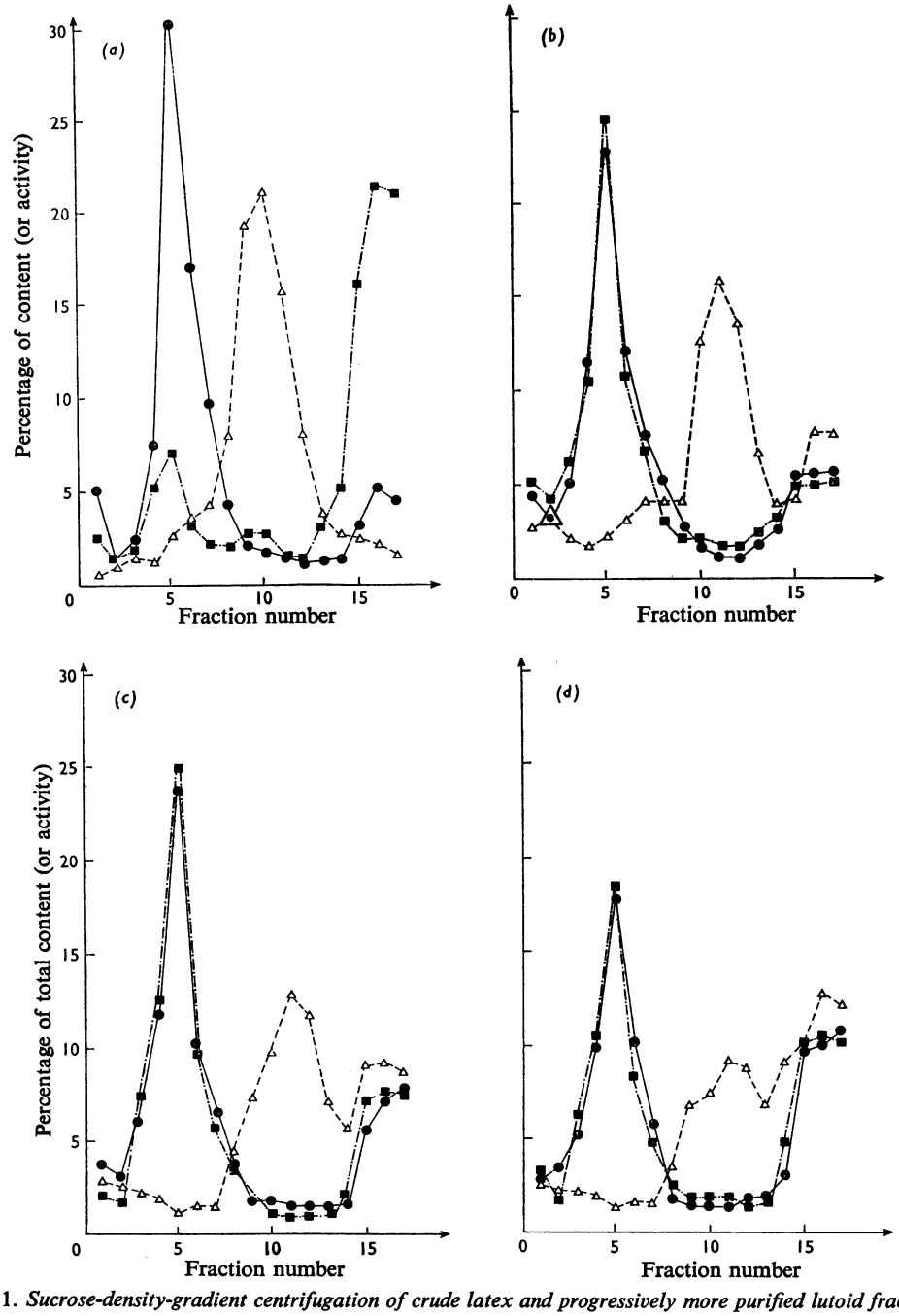

Fig. 1. *Sucrose-density-gradient centrifugation of crude latex and progressively more purified lutoid fractions*

The lutoid fraction was prepared from *H. brasiliensis* latex as described by Pujarniscle (1969). When being tapped, latex was diluted with 1–3vol. of 10mM-KCl–10mM-$MgCl_2$–0.3M-mannitol–0.1M-Tris–HCl buffer, adjusted to pH7.3. After centrifugation at 26500*g* for 30min at 4°C, the sediment was resuspended and washed with Tris–mannitol buffer under the same conditions. The final pellet, diluted with the same buffer, was layered on a continuous 0–50% (w/v) sucrose density gradient formed over 3ml of 75% (w/v) sucrose in Tris–mannitol buffer. Gradients were centrifuged at 90137*g* for 60min at 4°C. The lutoid band was collected and further purified by at least two isopycnic centrifugations under the same conditions. Each 2ml fraction was assayed for RNA content and acid phosphatase and polyphenol oxidase activities. RNA content (■) was determined by the method of Tupy (1969). Acid phosphatase (●) and polyphenol oxidase (△) activities were determined by the method of Pujarniscle (1969). Results are presented as percentages of the total content (or activity) recovered. (*a*) Crude latex; (*b*) crude lutoid fraction; (*c*) lutoid fraction after a first purification by a sucrose-gradient centrifugation; (*d*) lutoid fraction after a second purification by sucrose-gradient centrifugation.

will require further experimentation. Some preliminary results indicate that RNA extracted from this fraction and fractionated by 2.4% (w/v) polyacrylamide-gel electrophoresis is resolved into several main components including constitutive rRNA fractions and some very interesting degraded rRNA fractions migrating more slowly than tRNA (B. Marin, P. Trouslot & S. Pujarniscle, unpublished work). This intact RNA represents 2–3% of the total latex RNA whereas serum rRNA constitutes 4–5% of the total RNA. These results, however, need to be confirmed.

Latex is always contaminated by bacteria during tapping (Taysum, 1961) and the RNA found could originate from such contaminating bacteria. However, our estimations of the extent of bacterial contamination account for less than 1% of the recovered RNA.

Acid phosphatase is a marker enzyme of the lutoid organelle and for this reason it has been suggested that these organelles must be lysosomes (Pujarniscle, 1969). As lysosomes contain acid ribonuclease and phosphodiesterase activities (Pujarniscle, 1969), it would not be surprising if lutoid RNA consists of, in part, degraded RNA molecules from other cellular compartments. On the other hand, it has also been reported that the lysosomal fractions from other organisms contained intact ribosomes (Moor & Muhlethaler, 1963; Matile, 1969; Pitt & Galpin, 1973). Since lutoid organelles are easily purified, they appear to be a very convenient material in which to investigate the rather debatable question of the presence of ribosomes in lysosomes.

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