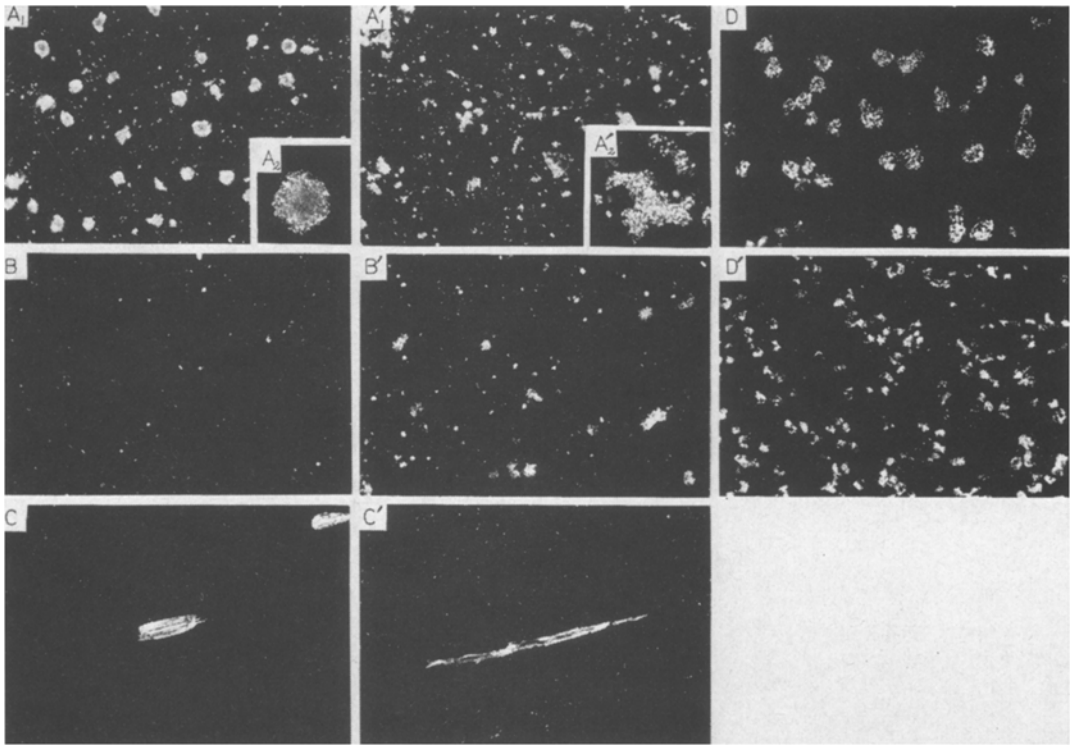


Pattern of calcium oxalate crystals. *Nicotiana tabacum* Linn., A_1, healthy leaf; A_1', affected leaf; ×100. *N. tabacum* Linn., A_2, healthy leaf; A_2', affected leaf; ×400. *Nicotiana rustica* Linn., B, healthy leaf; B′, affected leaf; ×100. *Dendrobium*, C, healthy leaf; C′, affected leaf; ×100. *Beta vulgaris* var. *cicla*, D, healthy leaf; D′, affected leaf; ×100.

crystalline inorganic components markedly different from that in a healthy plant of the same kind, it is safe to conclude that at least some abnormality is present in the metabolic system of substances in that plant. 3. The pattern of crystalline inorganic components in the host plant can become an important indicator for probing the behavior of a virus in the host plant. Consequently, the pattern analysis of crystalline inorganic components may be expected to make a considerable contribution in the future for the elucidation of the proliferation mechanism of viruses and formation mechanism of crystalline inorganic components.

Poly(A) Associated RNA from Mitochondria and Microsomes of Rat Brain

A. CUPELLO[1] and G. ROSADINI

Centro di studio per la Neurofisiologia Cerebrale, Consiglio Nazionale delle Ricerche, Ospedale S. Martino, Genova (Italy), 19 January 1976.

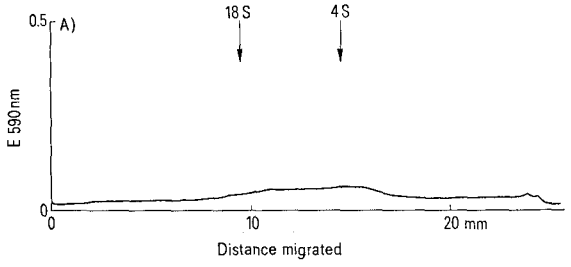
Summary. Rat brain mitochondria contain a significant proportion of poly(A) associated RNA which is higher than that found in microsomes from the same source. When steady state poly(A) RNA of brain mitochondria was analyzed by microelectrophoresis, it displayed a characteristic separation pattern with a large amount of 'free' poly(A).

Most of eukaryotic messenger RNA's appear to be associated with a polyadenylate stretch at their 3′ end[2–5]. Whereas the function of this poly(A) 'tail' is not completely understood, it gives an important tool for mRNA isolation. In fact, poly(A) associated RNA may be isolated binding it specifically, in condition of high ionic strength, to Millipore filters[6], olygo(dT)-cellulose[7] or poly(U)-sepharose[8].

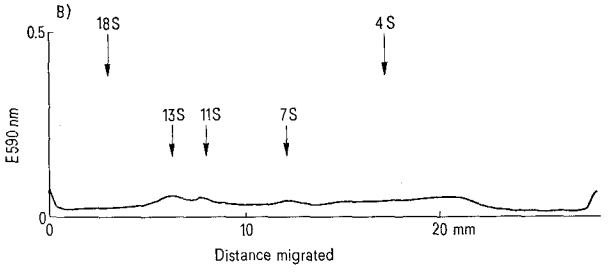
Recently the poly(A) associated fraction of RNA from rat brain polysomes was shown to be very active in an in vitro protein synthesis system[9]. On the other hand, microsomal poly(A) RNA isolated from the brain of myelinating rats can direct the synthesis of rat myelin encephalitogenic protein[10]. These results indicate that also the brain polysomal poly(A) associated RNA has the properties of messenger RNA.

Poly(A) associated RNA was detected also in mitochondria of HeLa cells[11,12] and thoroughly analyzed for sedimentation and electrophoretic characteristics[13]. As far as we are aware, no study on poly(A) RNA in brain mitochondria is at present available. Thus we studied poly(A)-associated RNA binding to Millipore filters in mitochondria of rat brain with regard to its amount and electrophoretic characteristics. We studied as well for comparison

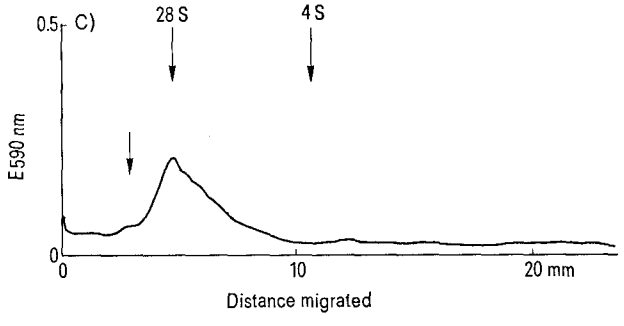
Pattern of separation of Millipore filter binding RNA from brain microsomes and mitochondria on 1.7% acrylamide —0.7% agarose and 3.5% acrylamide —0.7% agarose gels. About 0.01 o.d.u.$_{260}$ were applied. The gels were run at 6 °C under 10 volts/cm with an average current of 200 μA/gel, 20′ for the 1.7% and 30′ for the 3.5% gel.



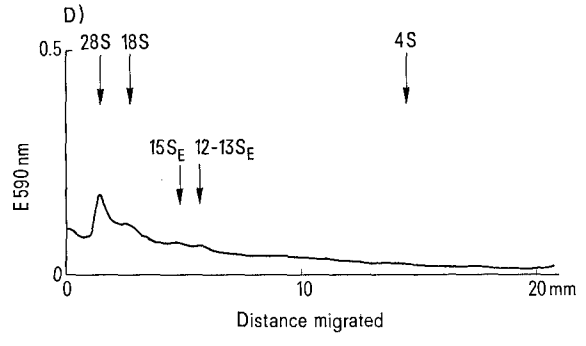
A) Poly(A) associated RNA from brain mitochondria run on a 1.7% acrylamide —0.7% agarose gel.



B) Poly(A) associated RNA from brain mitochondria run on a 3.5% acrylamide —0.7% agarose gel.



C) Poly(A) associated RNA from brain microsomes run on a 1.7% acrylamide —0.7% agarose gel.



D) Poly(A) associated RNA from brain microsomes run on a 3.5% acrylamide —0.7% agarose gel.

the characteristics of poly(A) RNA from brain microsomes.

We have chosen, as a tool for poly(A) RNA isolation, the Millipore filter technique because it is simple and suitable for isolation of poly(A) tails of more than 50 nucleotides long, as appears to be the case for mitochondrial poly(A)[13].

For the electrophoretic analysis of the Millipore binding RNA, we have used a micromethod which allows analysis of RNA in 0.01 o.d.u. 260 amounts[14]. This enabled us to analyze unlabelled steady state poly(A) RNA of brain mitochondria starting from a reasonable number of animals (4 rats for each experiment).

Methods. Preparation of subcellular fractions and extraction of RNA. Microsomes and mitochondria were prepared from groups of 4 rats of around 250 g weight by the procedure described previously[14]. RNA was extracted from the 2 fractions by the methods described in the same paper.

Adsorption of RNA on Millipore filters. After one wash by alcoholic reprecipitation, RNA was dissolved in 1 ml of 500 m*M* KCl, 1 m*M* $MgCl_2$, 10 m*M* *Tris* (pH 7.6)[6]. 2 aliquots of 0.1 ml were taken for the determination of the optical density of the solution at 260 nm before the absorption on Millipore (o.d. ^{1260}) and the remaining 0.8 ml were passed at 10 °C at the speed of 0.5 ml/min through a Millipore filter presoaked in the same solution, according to the technique of BRAWERMAN et al.[6]. The filter was then washed 3 times with 1 ml of the buffer. We assessed previously that in the 3rd wash virtually no more material is washed from the filter. All the filtrates were collected and the final solution was brought to exactly 5 ml and read at 260 nm in the spectrophotometer (o.d. ^{2260}). The percentage of the input RNA retained on the filter was calculated by the following formula:

$$\frac{0.8 \times \text{o.d.}^1_{260} - 5 \times \text{o.d.}^2_{260}}{0.8 \times \text{o.d.}^1_{260}}$$

The filters were then kept at —80 °C until the RNA elution step.

Elution and electrophoresis of Millipore binding RNA. The elution of bound RNA from the filters was performed according to the procedure of BRAWERMAN et al.[6]. After the elution, RNA was precipitated by ethanol and redissolved in sample buffer[15] at a o.d.$_{260}$ of 10.0.

[1] Present address: Institute of Neurobiology, Medical Faculty, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden.

[2] L. LIM and E. S. CANELLAKIS, Nature, Lond. *227*, 710 (1970).
[3] J. E. DARNELL, R. WALL and R. J. TUSHINSKI, Proc. natn. Acad. Sci., USA *68*, 1321 (1971).
[4] S. Y. LEE, J. MENDECKI and G. BRAWERMAN, Proc. natn. Acad. Sci. USA *68*, 1334 (1971).
[5] J. MENDECKI, S. Y. LEE and G. BRAWERMAN, Biochemistry *11*, 792 (1972).
[6] G. BRAWERMAN, J. MENDECKI and S. Y. LEE, Biochemistry *11*, 637 (1972).
[7] H. AVIV and P. LEDER, Proc. natn. Acad. Sci. USA *69*, 1408 (1972).
[8] M. ADESNIK, H. M. SALDETT, W. THOMAS and J. E. DARNELL, J. molec. Biol. *68*, 21 (1972).
[9] I. DE LARCO, A. ABRAMOWITZ, K. BROMWELL and G. GUROFF, J. Neurochem. *24*, 215 (1975).
[10] L. LIM, W. BERTHOLD, J. WHITE, C. HALL and A. N. DAVISON, Abstr. V. Meeting of International Society for Neurochemistry Barcelona 2–6 Sept. 1975, p. 279.
[11] S. PERLMAN, H. T. ABELSON and S. PENMAN, Proc. natn. Acad. Sci., USA *70*, 350 (1973).
[12] D. OJALA and G. ATTARDI, J. molec. Biol. *82*, 151 (1974).
[13] D. OJALA and G. ATTARDI, Proc. natn. Acad. Sci., USA *71*, 563 (1974).
[14] A. CUPELLO and H. HYDÉN, J. Neurochem. *25*, 399 (1975).

Percentage of Millipore filter binding RNA in some rat brain cytoplasmic fractions

Fraction	No. of experiments	%
Microsomes	7	5.8 ± 0.9
Mitochondria	7	7.7 ± 1.7

RNA was fractionated by microelectrophoresis in 0.01 o.d.u.$_{260}$ amounts on a) 1.7% acrylamide −0.7% agarose gels for analysis of the whole range of RNA molecules (from 2.4×10^4 to 10×10^6 Daltons), b) 3.5% acrylamide −0.7% agarose for analysis of RNA in the low molecular weight range, according to a procedure described previously[15].

Calculation of the S_E and molecular weight values from electrophoretic data. The S_E values were calculated on the basis of a linear relationship between log S_E and mobility. Analogously, the M.W. values were calculated assuming a linear relationship between log MW and mobility. The reference values were provided by 4S, 18S and 28S RNA for the 1.7% acrylamide gel and by 4S and 18S RNA for the 3.5% gel. When such bands were not clearly distinguishable on the poly(A) associated RNA pattern their positions were taken from a parallel gel run with a sample of microsomal RNA.

Results and discussion. A certain proportion of brain mitochondrial RNA appears to be associated with a poly(A) stretch as can be seen by its binding to Millipore filters in a buffer of high ionic strength. Moreover, such a proportion appears to be slightly higher than in brain microsomes (Table).

Poly(A) associated RNA from brain mitochondria was fractionated by microelectrophoresis on agarose-acrylamide gels as reported in Figures A and B. On a large pore gel, such RNA appears to be rather heterogeneously distributed between some 14 S_E and something lower than 4 S_E with a small peak at about 13 S_E (Figure A). In order to have a better resolution, this RNA was run on a more concentrated gel as shown in Figure B. Here we could resolve two discrete species with S_E of about 13 and 11, a rather broad peak at 7 S_E and a heterogeneous band with its maximum at an S_E value slightly lower than 4.

Figure C and D show the fractionation patterns of poly(A) RNA from brain microsomes on the large and small pore gels. This RNA presents a heterogeneous distribution ranging from some 8 to about 30 S_E with in addition a small band of high molecular weight (5.0×10^6 Daltons), as shown in Figure C. When it was fractionated on a 3.5% acrylamide −0.7% agarose gel, allowing a higher resolution in the 4S–18S range, we could recognize 2 low molecular weight bands with S_E values of 15 and 12–13.

These results show that a significant proportion of poly(A) associated RNA is present in brain mitochondria; it does not originate entirely from microsomal contamination as can be inferred from its higher concentration and its distribution which, ranging from some 14 to about 4 S_E (Figure A), is quite different from that of its microsomal counterpart. Its specific electrophoretic pattern also differentiates it clearly from total mitochondrial RNA whose distribution is completely different on the 2 types of gels considered here.

The fact that mitochondrial poly(A) RNA of the brain ranges from 14 to 4 S_E is in reasonable agreement with the findings of OJALA and ATTARDI[13], who found that pulse labelled poly(A) RNA from HeLa cell mitochondria is smaller than 12 S when analyzed under denaturing conditions. However, 3 discrete bands with S_E values of 7, 11 and 13 could be detected (Figure B) where the first of them seems to correspond to the major discrete band found by OJALA and ATTARDI[13].

Moreover, in our preparation, which concerns steady state poly(A) RNA from whole mitochondria, there is a large amount of low molecular weight material peaking at an S_E value slightly lower than 4 (Figure B). This material does not seem to correspond to breakdown artifacts because of its absence in the corresponding preparation from microsomes and its high reproducibility. It is more probably 'free' mitochondrial poly(A) which was detected previously in mitochondria from HeLa cells and which was described to migrate a little faster than 4 S mitochondrial transfer RNA[12].

The Millipore binding RNA from brain microsomes, presenting a heterogeneous distribution between 8 and 30 S_E (Figure C), appears to be analogous to the RNA which can be isolated by the same technique from mouse sarcoma polysomes[6]. Microsomal Millipore binding RNA from brain present also some discrete bands, 2 of low molecular weight (12–13 and 15 S_E) and 1 of quite high molecular weight (5.0×10^6 Daltons).

[15] A. CUPELLO and H. HYDÉN, Neurobiology *5*, 129 (1975).

Wall Hydroxyproline and Growth of Georeactive Roots (*Zea mays* L.)

M. C. JOTTERAND-DOLIVO and P. E. PILET

Institut de Biologie et Physiologie végétales de l'Université, Place de la Riponne, CH-1005 Lausanne (Switzerland)
25 February 1976.

Summary. For the growing maize roots the increase of the content of wall hydroxyproline was related to a decrease of the root elongation and vice-versa. For the georeactive roots the wall hydroxyproline level was lower in the upper part which elongates more than the lower part containing more wall hydroxyproline.

It has been largely demonstrated that proteins, characterized by a high level in hydroxyproline (OH-PRO), are firmly associated with cellulose microfibrils in plant cell walls[1]. The nature of these proteins is still unknwon but a few papers have demonstrated a correlation between the increase of the OH-PRO content of the wall and the decrease of cell elongation[2–5]. The loss by the wall of its ability to extend might be due to a rise in the OH-PRO-O-arabinoside glycopeptides crosslinkages with the cellulosic framework[1]; but the direct intervention of OH-PRO as the strengthening agent of the cell wall has not yet been clearly proved[6].