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).

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

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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].

The aim of the present experiments was to investigate the wall OH-PRO content for the upper and lower halves of geotropical maize roots. The downward curvature of these roots arises from the extension rate of the lower part being depressed much more than that of the upper part[7,8]. Such asymmetrical growth has to be related to the production, by the root cap, of some growth inhibitors (see[9]) which move basipetally from the tip to the extending zone of root[10,11] and also laterally inside the horizontal root apex[12]. On the other hand, the gradients of the wall OH-PRO were previously reported for similar maize roots when growing in vertical position[13].

Selected caryopses of *Zea mays* L. cv. Kelvedon 33 were grown in darkness (22°). When the primary roots, which elongated vertically, reached 15 ± 3 mm, the 10 ± 0.2 mm apical segments were cut and mounted on plastic frames with their basal cut ends covered with moist buffered (pH 6.1) filter paper. The frames were placed in closed boxes (22 ± 1°) in which a humid atmosphere (90 ± 5%) was maintained. All segments were kept in white light (0.9 ± 6.10^{-2} J m^{-2} s^{-1}). Elongation was recorded by making shadow photographs magnified 3.5 times; the standard error assessed by the *t*-test were not higher than 12%. All technical data were discussed elsewhere[14]. Wall OH-PRO content was determined after 14 h of horizontal (or vertical) presentation of the root segments. The georeactive part was carefully cut into upper and lower halves and the corresponding zone of the control (vertical roots) was also prepared by cutting 2 equal parts. The cell wall fraction was obtained by the technique proposed by RIDGE and OSBORNE[3] and OH-PRO was analyzed, according to BERGMAN and LOXLEY[15], in neutralized and concentrated hydrolysates[13].

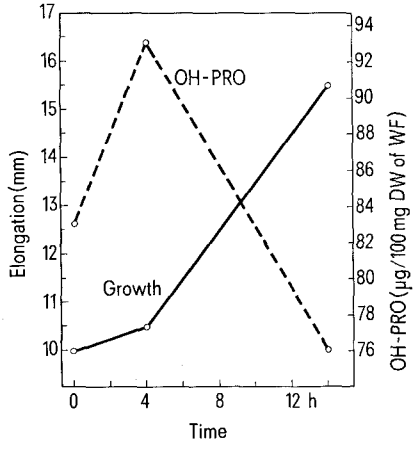

Fig. 1. Elongation (in mm) and wall OH-PRO content (in µg per 100 mg of the wall fraction dry weight) of the extending zone (corresponding to the bending part of similar georeactive roots) of vertical maize roots.

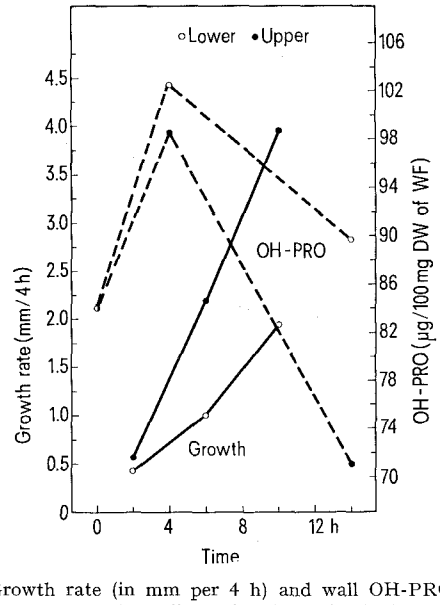

Fig. 2. Growth rate (in mm per 4 h) and wall OH-PRO content (in µg per 100 mg of the wall fraction dry weight) of the upper and the lower halves of the georeactive zone of maize root segments, placed (at 0 h) in horizontal position.

Results, related to the rectilinear growing root segment, are reported in Figure 1. As can be seen, during the first 4 h, a low initial elongation occurs, followed (at least for the next 10 h) by a rapidly increasing growth. The first step observed – which could be a preparatory phase – corresponds to an increase of the wall OH-PRO level. Then OH-PRO content significantly decreases when the growth rate increases. Quite similar results were obtained for the upper and lower halves of the bending zone of the geotropically stimulated root segments; they are given in Figure 2. It can be noticed that the upper part of root segments, for which extension is higher, contains less OH-PRO than the lower halves which elongates less. For both root parts tested, the increased growth rate exactly corresponds to a significant decrease on the OH-PRO level. This has to be related to the size changes in the parenchyma cells – directly dependant on the inhibiting substances formed in the root cap[9,11] – which are increasing more rapidly in the upper part of the bending zone than in the lower[16].

In conclusion, the inverse correlation between wall OH-PRO content and growth of root cells is strictly fullfilled. However, such a statement is not so clear for the root segments analyzed during the first hours of their elongation period. But this preparatory phase has to be discussed critically: cutting and mounting the segments may have some immediate traumatic consequences and the adaptation of this plant material to the new growing conditions has also to be considered. Some general questions have still to be solved. Is OH-PRO itself directly implicated in the extensibility of the wall, or is the whole wall glycoprotein concerned? Is OH-PRO accumulation due to an increased formation of one wall compound, or to the synthesis of some new OH-PRO-rich-glycopeptide which specifically inhibits the wall extension? During this wall extension, which acid-labile linkages are modified? and is OH-PRO participating in some of these cross-bridges? All these questions will remained unanswered until the wall glycoprotein can be isolated and entirely characterized.

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