Polish Journal of Environmental Studies Vol. 6, No. 3 (1997), 35–40

Effects of Calcium and Toxic Metals on RNA Synthesis and Transport in Cortex Cells of Root Meristem of *Pisum Sativum* L.

B. Gabara[1], M. Krajewska
Department of Plant Cytology and Cytochemistry, University of Lodz,
Banacha 12/16, 90-237 Lodz, Poland

Received 29 October, 1996
Accepted 19 February, 1997

Abstract

Experiments with ^{3}H uridine incorporation showed that a 144-hr treatment with cadmium, chromium or lead, each at a concentration of 10^{-4} M, as well as the addition of calcium (10^{-3} M), affected RNA in the nucleolus, nucleoplasm and cytoplasm in cortex cells of pea root meristem. Disturbances in RNA transport (especially from the nucleolus to the cytoplasm) appearing due to toxic metals and calcium suggest that this RNA was mostly of nucleolar origin.

Keywords: RNA synthesis and transport, metals, calcium, root, *Pisum sativum* L.

Introduction

Agricultural processes such as the exessive use of fertilizers, liming and sewage sludge have resulted in contamination of agricultural soil with toxic metals. These metals can be passively taken up and moved into the food chain.

Numerous studies on the effect of toxic metals on plants have been reported [2, 12, 21, 25, 29] showing that metals act on the plasmalemma, nucleus, mitochondria and chloroplasts (after [27]). However, the mechanisms of their toxicity are still far from clear.

In order to better understand the action of metals at the cellular level, we described the changes in dry mass of the nucleoplasm and the cytoplasm [6,23], in terms of DNA and NYS-protein contents [32] as well as in terms of DNA synthesis [7], mitotic index and chromosomal aberrations [5]. These studies were undertaken in pea roots growing in the presence of cadmium, chromium or lead. Moreover, since toxic effects caused by metals may be ameliorated after calcium addition [5,6,7,9,10,30], we wanted to check whether calcium will diminish the effect of metals on the uptake of uridine into nuclear, nucleolar and cytoplasmic RNA.

Therefore, the present studies were undertaken to examine RNA synthesis and transport in pea root cells growing in the presence of toxic metals with or without the addition of calcium.

Material and Methods

Cortical cells of the root meristem of *Pisum sativum* L. seedlings growing in the presence of calcium and/or heavy metals were the object of the present studies. Seeds were washed in tap then distilled water and were imbibed in the following solutions: calcium ($CaCl_2$), 10^{-3}M, and/or cadmium ($CdCl_2$), chromium [$Cr_2(SO_4)_3 \times 6H_2O$] or lead ($PbCl_2$), all 10^{-4}M. After 24 hr, they were sown on cotton wool moistened with the same solutions that they were imbibed with, and cultivated as described earlier [23].

To study RNA synthesis, roots of six-day-old seedlings were incubated in [5–^{3}H] uridine at 20 μCi/ml (spec.activity 20 Ci/mM) for 2 hr. Moreover, to check the tested jons' action on RNA transport, roots were incubated in radioactive uridine solution for 20 min and post-incubated in non-radioactive precursor for 40 min.

The meristematic zones of rots (1 mm from the cap junction) were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 3 hr, washed in buffer and post-fixed in 1% osmium tetroxide in the same buffer for 2 h. After dehydration in ethanol, the material was embedded in Epon 812. Longitudinal sections (1 μm) were cut on an Ultratome LKB and adhered to glass slides, dipped previously in gelatin. They were coated with liguid Ilford K2 emulsion, and were exposed at 00C for 21 days. After developing and fixation, the material was stained with 0.1% toluidine blue.

[1] Corresponding author, Fax +048 42 35 44 23

The results were expressed as the number of silver grains per nucleolus, nucleoplasm and cytoplasm of the cortex cells from the root meristem. The area of the nuclei and nucleoli were calculated by the formula πr^2 or $\pi r_1 r_2$ (r - diameter) in the case of elliptic nuclei. For cell area, a x b (length, width) was used since most of the cells were rectangular in longitudinal sections of the root meristem. One hundred cells per root, and 10 roots per treatment were taken in two replicates. To count the background grains, a micrometric ocular with grid and scale was employed, where necessary.The results were analyzed statistically.

Results

RNA Synthesis

The tested metals influenced the incorporation of ^{3}H uridine into the nucleolus, nucleus and cytoplasm of pea root cells in different ways (Fig.1). A drastic reduction in radioactivity was observed in cells treated with cadmium (Figs. 1-2), while in the lead-treated material an increase in RNA synthesis in the nucleolus occurred and a diminution in the radioactivity of the nucleus, but most of the cytoplasm was seen. The presence of chromium reduced cytoplasmic labelling in spite of RNA synthesis levels in the nucleolus and nucleoplasm being similar to those in the control material (Fig.2).

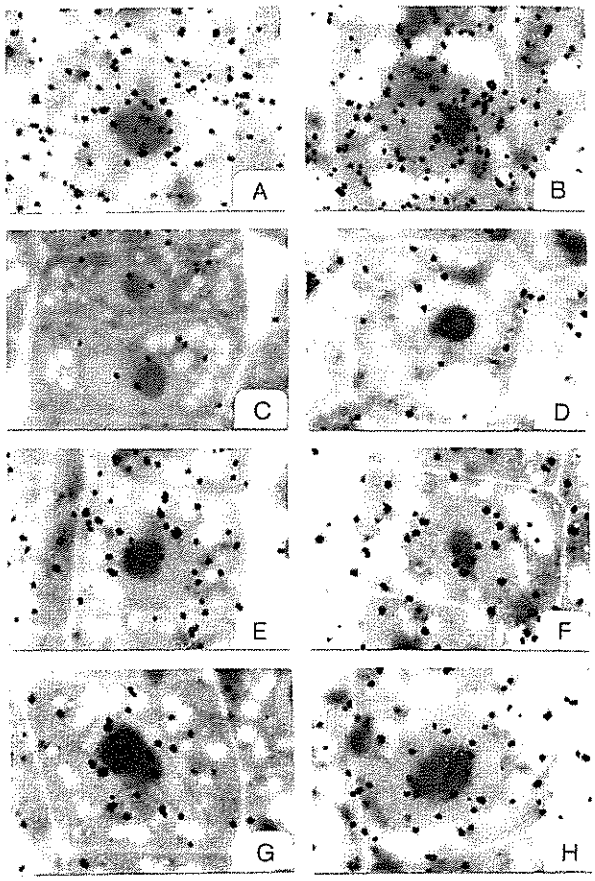


Fig. 1. Incorporation of ^{3}H uridine into cortex cells of pea root meristem growing in: (A) water and solution of: (B) calcium, (C) cadmium, (E) chromium, (G) lead, and in the mixture of calcium with (D) cadmium, (F) chromium and (H) lead. x 2000.

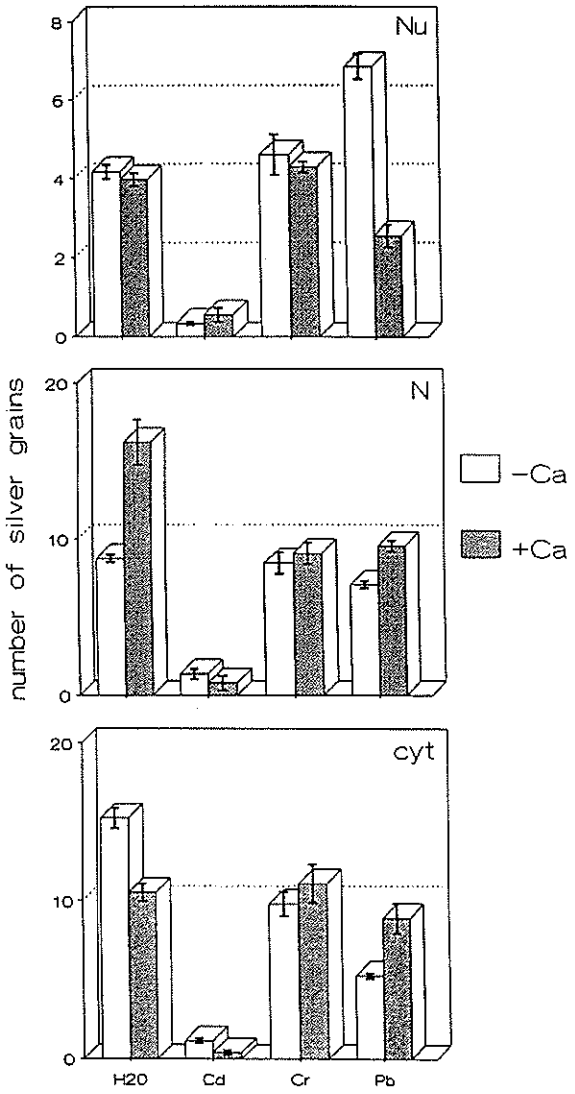


Fig. 2. Radioactivity of pea root meristematic cortex cells treated with heavy metals and/ or calcium after 2 hr incubation with ^{3}H uridine expressed as a number of silver grains per 10 μm^2 surface area of nucleolus, and 100 μm^2 surface area of nucleoplasm and cytoplasm. Means ± SE of ten roots, with two replicates are given (n=20).

The above results indicate that toxicity of the tested metals is as follows: Cd>Pb>Cr.

Calcium Effect

Although calcium alone caused strong stimulation of RNA synthesis in the nucleoplasm, when this ion was added to each test solution containing metals it changed the radioactivity of the nucleus, nucleolus and cytoplasm in different ways (Figs.1-2). Calcium in the lead solution reduced RNA synthesis in the nucleolus and increased radioactivity of the cytoplasm (Fig.2). The addition of calcium to the other tested metals

changed neither RNA synthesis in the nucleolus and nucleus nor cytoplasmic radioactivity.

Transport of RNA

Analysis of autoradiograms carried out after a 20-min incubation with ^{3}H uridine showed the reduction in labelling of the nucleolus and especially cytoplasm (Fig.3A) in the material treated with cadmium. In the presence of lead, radioactivity of nucleolus diminished while radioactivity of the nucleus and cytoplasm increased. The presence of chromium did not change the radioactivity of the nucleus, nucleolus or cytoplasm.

The intensity of labelling of a single cell (nucleolus, nucleus and cytoplasm) increased after a 40-min postincubation, as compared with the series fixed after a 20-min incubation with ^{3}H uridine (Fig. 3B). During this period, continued incorporation of ^{3}H uridine into RNA must have taken place from the pool of precursors taken up by the plant during a 20-min incubation. Therefore, data in Fig.3 represent the results of both synthesis and migration of RNA, which occured during the post-incubation period. Comparative analysis of these autoradiograms has shown that besides cadmium, the presence of chromium also diminished radioactivity of the nucleolus, nucleoplasm and cytoplasm after a 40-min post-incubation. Moreover, in the lead-treated material, the radioactivity of the nucleolus was similar to that of the control. The labelling of the cytoplasm decreased in comparison to control after both a 20-min incubation and post-incubation period (Fig. 3B).

Disturbances in RNA transport from the nucleoplasm but mostly from the nucleolus to the cytoplasm were distinctly visible when labelling the cell (nucleus, nucleolus and cytoplasm) was expressed as 100% (Fig.4). These data show that such disturbances also occurred in the presence of lead.

Calcium Effect

Calcium alone caused a reduction in the radioactivity of the nucleus and nucleolus but did not change that of the cytoplasm after a 20-min incubation with ^{3}H uridine (Fig. 3A). Adding calcium to tested metal solutions diminished the intensity of labelling of the nucleus, nucleolus and cytoplasm after a 20-min incubation (Fig. 3A). This was quite evident in the chromium and lead solution. Only in the presence of cadmium did an increase in radioactivity of the nucleolus take place.

After 20-min of ^{3}H uridine followed by 40-min post-incubation in non -radioactive uridine the radioactivity of the nucleus, nucleolus and cytoplasm in the calcium-treated material underwent reduction in comparison to labelling in the material growing without calcium (Fig. 3B) although an increase in the number of silver grains nucleus and nucleolus and radioactivity of the cytoplasm was observed (as compared with 20-min of incubation). Calcium added to the cadmium and chromium solutions caused an increase in radioactivity of the nucleolus while in cadmium and lead presence it diminished labelling of the nucleus. Calcium alone as well as in the cadmium and lead solution

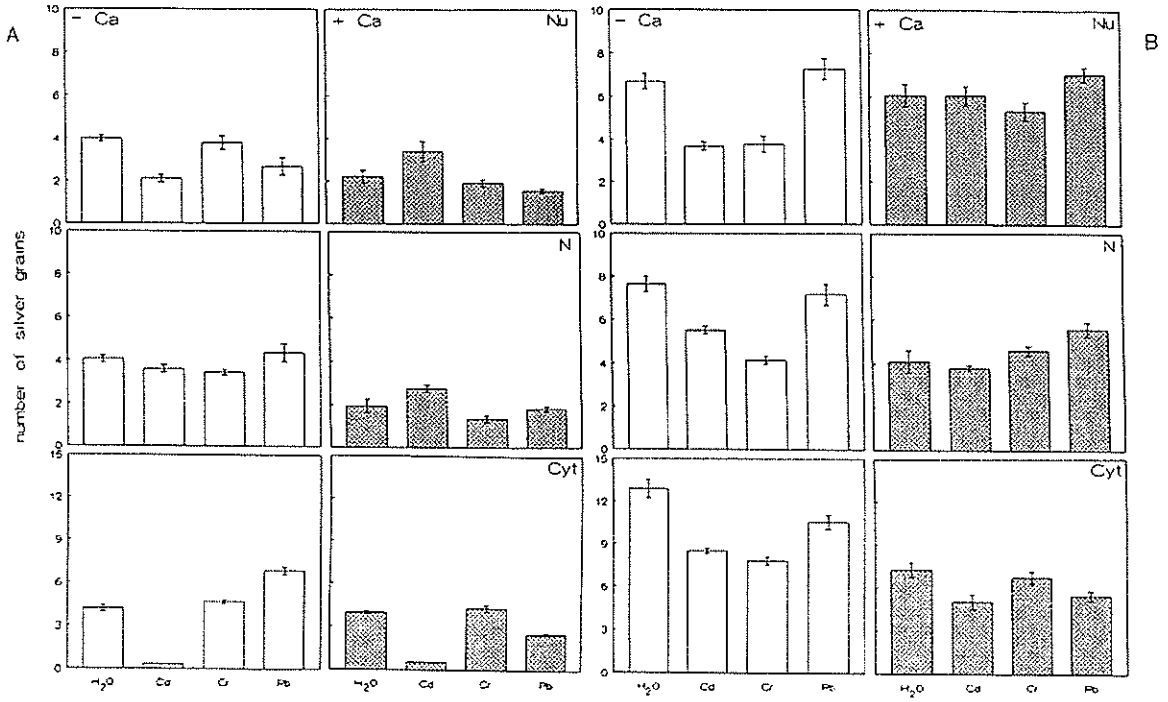


Fig. 3. Incorporation of ^{3}H uridine into nucleolus (Nu), nucleoplasm (N) and cytoplasm (Cyt) after: A/ a 20-min incubation with ^{3}H uridine and B/ a 40-min post-incubation in non-radioactive uridine following 20-min incubation in radioactive uridine. The number of silver grains was calculated per profile of nucleolus, nucleoplasm and cytoplasm. Mean ± SE of ten roots, with two replicates are given (n=20)

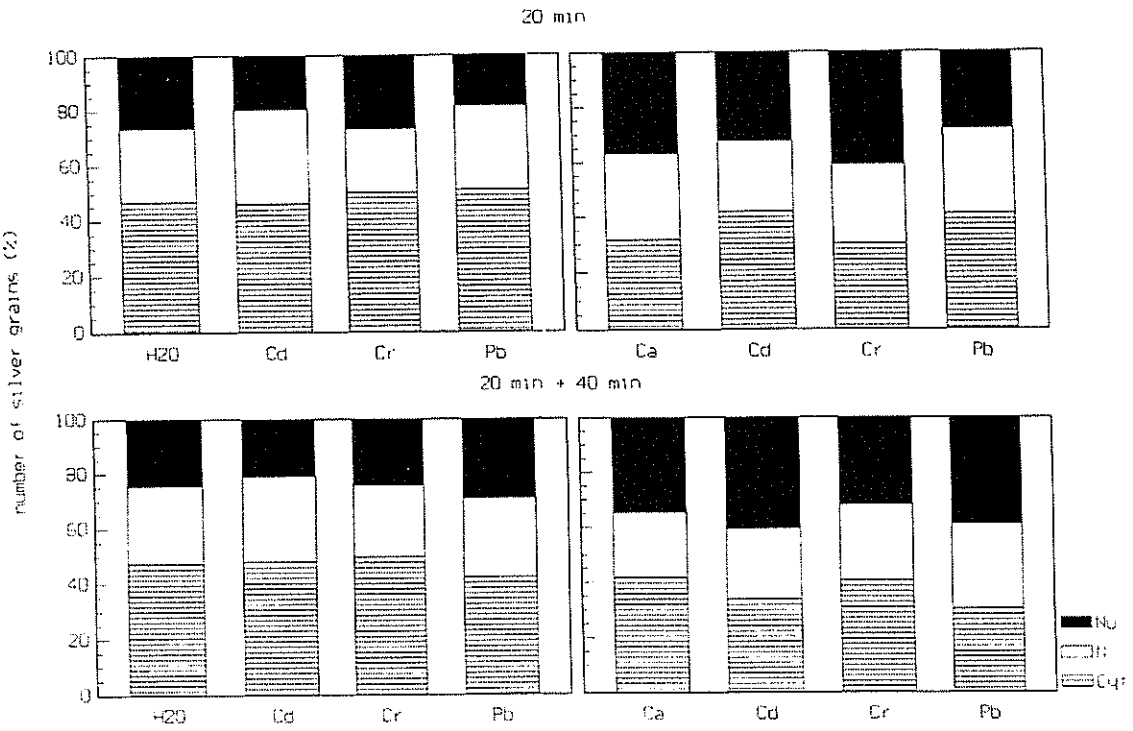


Fig. 4. Nucleolar, nucleoplasmic and cytoplasmic radioactivity expressed as 100% of a total radioactivity of cells after a 20-min incubation with ^{3}H uridine (upper diagrams) and after a period comprising 20-min incubation and 40-min post-incubation (lower diagrams). Values are means of 2 experiments.

reduced RNA migration from the nucleolus to the cytoplasm (Fig .4). Only when calcium was with chromium did a slight increase in RNA transport from the nucleolus to the cytoplasm take place.

Discussion

The results of the present experiments show that metals (Cd,Cr,Pb) affected RNA sythesis in cortex cells of the root meristem of *Pisum sativum* L. Among the most toxic was cadmium and the least toxic chromium. Only in the presence of lead was a stimulation of RNA synthesis in the nucleolus observed. Contradictary results of metals effects on RNA synthesis were also described in *Lemna* and *Pisum*. While diminution in RNA synthesis accompanied by a fast decrease in RNA content in the presence of lead was demonstrated in *Lemna minor* (Schneider and Urlik, unpubl.), a higher RNA sythesis was showed in *Pisum sativum* [18]. However, enhanced RNA sythesis was observed mostly after a short time [18], even 1-hr treatment [14].

Partly correlated with the number of nucleoli [8], the intensity of RNA sythesis estimated on the basis of incorporation of radioactive RNA precursor is a derivative of the number of genes [13,16,17] and of the functional state of chromatin, expressed in the degree of chromatin condensation. Metals binding to phosphate groups of nucleotides and nucleic acids [28] may reduce the pool of substrates for nucleic acids synthesis [1, 26], and diminish template activity of chromatin due to its condensation [22]. By binding to SH - proteins [28], metals may also affect RNA polymerase I, responsible for ribosomal RNA synthesis [4, 11]. At the present moment it is difficult to indicate which of the mechanisms mentioned above could be responsible for the variations in RNA synthesis described in the present paper.

After 30 min, the majority of newly synthetized RNA is in a form of pre-rRNA precursors (after [15]) but not all cytoplasmic RNA represents rRNA. A certain of this RNA could be ascribed to mitochondria and plastids. Moreover, in the response to metal treatment the induction and accumulation of mRNA for so called shock proteins also took place [3, 31]. Disturbances in RNA transport from the nucleolus to cytoplasm reported in the present paper seem to indicate that this RNA is of nucleolar origin.

Since rRNA synthesis occurs mostly in the middle periods of G_1, S and G_2 and enhanced transport of rRNA arrives at the final periods of G_1, S and G_2 [19, 20], disturbances in RNA transport as well as its synthesis could result from changes in the number of cells in G_1, S and G_2 [24] caused by these toxic metals.

The results of the present studies did not confirm the positive role of calcium ions in diminishing the toxicity of heavy metals although such a calcium effect was well documented by many authors [5, 7, 8, 9, 10, 30]. This is in agreement with our earlier experiments on the pea root meristem demonstrating that calcium

added to the metal solution also caused a further reduction of dry mass of nuclei and its concentration in nuclei and cytoplasm [6] as well as of the content of DNA and NYS-protein [24], although this ion in used concentration ameliorated toxicity of metals as demonstrated a root length test (unpubl.). Calcium action seems to be complex since, at the concentration used in the present studies (10^{-3}M), it positively influenced root length, ameliorating metal toxicity (unpubl.), DNA synthesis [7], mitotic index [5], RNA synthesis in the nucleoplasm, and RNA migration to the cytoplasm; but diminished RNA synthesis in the nucleolus and reduced RNA transport from the nucleus to the cytoplasm.

In conclusion, our results permit us to state that in the cortex cells of the pea root meristem, calcium ions variously affect cellular processes deranged by heavy metals poisoning in different ways. The interpretation of such a complex situation clearly demands further study.

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