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Effects of Cd^{2+} on the nucleolus in root tip cells of *Allium cepa*

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Abstract — The effects of different concentrations of cadmium chloride ranging from 0.5 to 20 ppm on the nucleolus in root tip cells of *Allium cepa* were studied using the silver staining technique. The results indicated that after the treatment with Cd^{2+}, different changes in nucleolar morphology appeared. The nucleolar material was extruded from the nucleus into the cytoplasm, and the nucleoli at mitotic metaphase did not disappear. Apparently, cadmium showed a specific effects on the nucleoli in root tip cells of *Allium cepa*. The possible mechanism behind this phenomenon is also briefly discussed.

Keywords: cadmium chloride; *Allium cepa*; nucleolus.

1 Introduction

The content of Cd in the atmosphere and soil conspicuously increases as a result of industrial operations such as Zn smelting (Ernst, 1972; Takijema, 1973; Thornton, 1979). Generally speaking, Cd^{2+} at low concentrations is not toxic to plants, but at higher concentration Cd^{2+} is toxic and characteristically inhibits root growth and cell division in root tip cells of *Allium cepa* (Liu, 1992; Fiskesjö, 1988), induces leaf chlorosis accompanied by a lowering of photosynthetic rate (Bazzaz, 1974; Hampp, 1976; Huang, 1974; Bazynski, 1980), and impedes respiration (Lee, 1976), mitochondrial electron transport (Miller, 1973) and enzyme activities in *Phaseolus vulgaris* (Weigel, 1980).

It is common knowledge that silver impregnation is regarded as a specific stain of the nucleolus and the nucleolar organizing region (NOR). The silver staining technique has been widely applied in cytological studies to try to understand the nucleolar cycle and the nucleolar organization in both animals and plants. But the cytological research work on the toxic effects of Cd^{2+} on nucleolus in root tip cells

of *Allium cepa* using a silver staining technique has hardly ever been reported. The present study was designed to examine the effects of cadmium chloride on nucleoli in the root tip meristematic cells of *Allium cepa*.

2 Materials and methods

Healthy and uniform-sized bulbs of *Allium cepa* were selected for the present study for the advantage of their few ($2n=16$) and large chromosomes. The onion bulbs neither shooting of green leaves nor any growth of roots at the beginning of our experiments. The outer scales of the bulbs and the brownish bottom disc had been removed and the ring of the root primordia was left intact before the experiments started. Each experimental set were composed of twelve onion bulbs and ten best bulbs within each set were selected for the continued test (Fiskesjö, 1988).

The onions were placed directly in the solutions of cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) ranging from 0.5ppm to 20 ppm. The test liquids were changed regularly every day. Control roots were maintained in tap water. The bulbs were allowed to germinate producing roots in beakers for 24, 48 and 72h. For the cytological studies, silver staining sampling procedures were the same as thoses outlined by Liu (Liu, 1991). Some of the slides were also stained with methylene blue after the silver staining.

3 Results

Normally, the diploid nucleus of *Allium cepa* contains 1−2 rounded nucleoli (Fig. 1a). There are some changes in the nucleolar morphology after the treatment with different concentrations and durations of Cd^{2+}. Oval, elongated-oval dumb bell nucleoli were observed after 24 h treatments with 0.5 to 3 ppm Cd (Fig. 1b-c). The nucleoli became irregular in shape (Fig. 1d), nearly occupy the whole nuclei (Fig. 1c) and even became broken (Fig. 1e) as the concentration and duration of the treatment increased.

More pronounced changes were found after 48 h Cd-treatment. The nucleolar material was extruded from the nucleus into the cytoplasm. In Fig. 1f, nucleolar material was on the way from the nucleus into the cytoplasm after the 48 h treatment with 20 ppm Cd. Fig. 1 g-h showed that the silver-stained material in the form of several particulates irregularly distributed in the cytoplasm after increasing the concentration and duration of Cd^{2+} treatment.

Furthermore, in a few cells, the phenomenon we observed was that the nucleoli

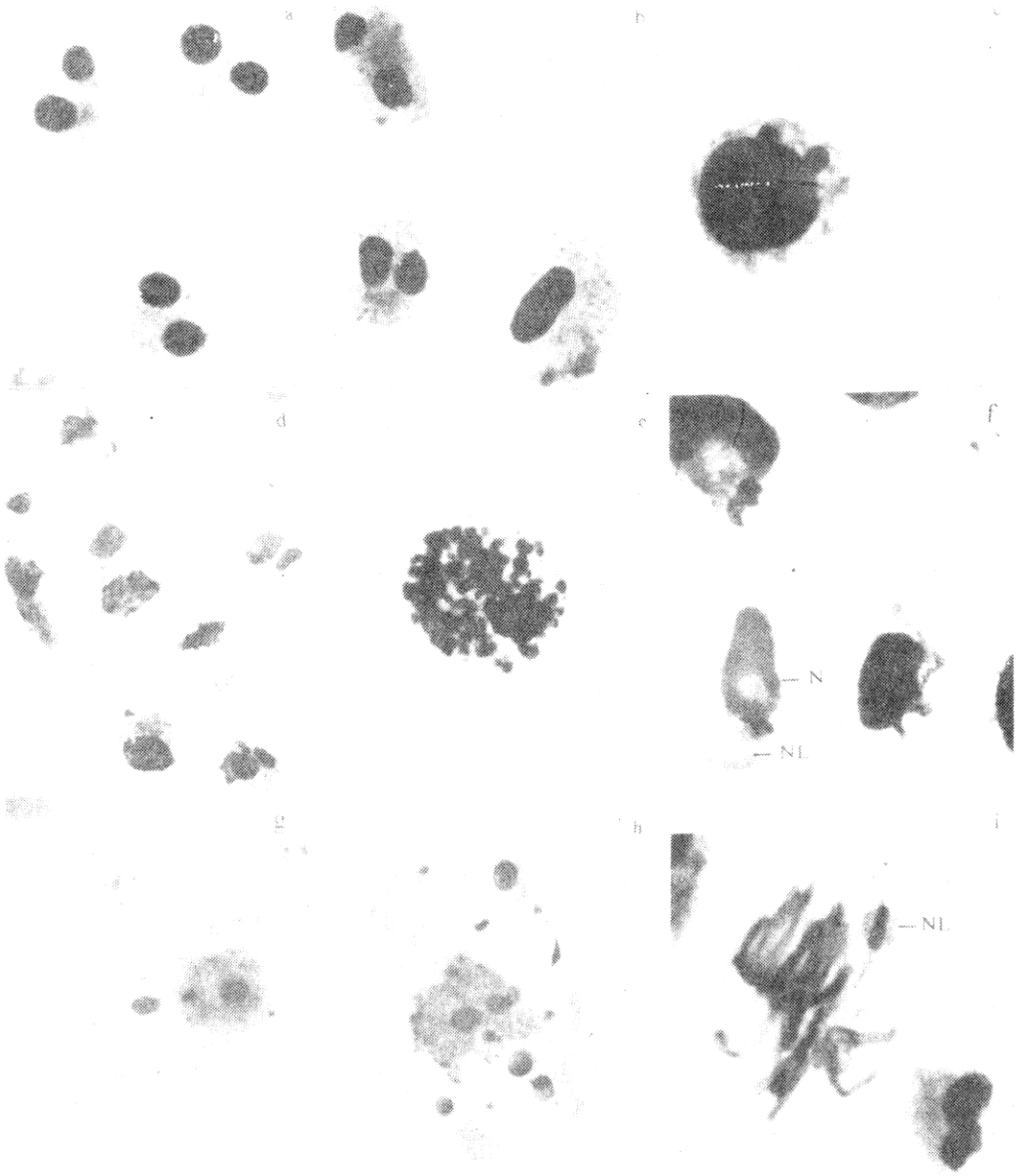

Fig. 1 The effects of Cd^{2+} on root tip meristematic cells of *Allium cepa*

a. Control cells (in tap water); b–c. 24h treatment with 0.5 to 3 ppm Cd; d. irregularily shaped nucleoli (3 ppm, 48h); e. broken nucleoli (3 ppm, 72h); f. nucleolar material on the way from nucleus to the cytoplasm (20 ppm, 48h); g–h. the nucleolar materials scattered in the cytoplasm (20 ppm, 72h); i. nucleoli appeared in metaphase (3 ppm, 48h); scale = 10 μm; N. nucleus; NL. nucleous

at mitotic metaphase did not disappear. The nucleoli were located on the either side or on each side of the equatorial plate (Fig. 1i). They did not link up with the chromosomes. The results of the present experiment is more or less than that of Liu *et al.* (Liu, 1988).

4 Discussion

Risueno *et al.* (Resueno, 1986) indicated that the nucleolus was a dynamic organelle whose structure and organization changed under different physiological and experimental conditions. This study is concerned with the silver-staining properties of the nucleoli in meristematic root cells of *Allium cepa* after the treatments with different concentrations of Cd^{2+}. Our results revealed that the silver-stained material was extruded from the nucleus into the cytoplasm. We think that all the silver-stained materials were of nuclear origin since they showed the same silver-staining reaction as nucleoli did. The results of the present experiments were more or less the same as those observed in the root tip cells of *Allium cepa* after the treatment with Al (Liu, 1991), with a little differences. For example, there were differences in the shape and position of the extruded and accumulated materials in cytoplasma after the treatments with Cd and Al. As for the phenomenon that nucleoli metaphase at mitotic did not disappear, the result coincides with the finding of Liu *et al.* (Liu, 1988).

It is well known that the nucleolus is the metabolic center of RNA. The integrity of the nucleolus depends on the existence of Ca^{2+} (Wang, 1988). Wen *et al.* (Wen, 1989) stated that Ca^{2+} could be replaced by Cd^{2+}, because they had similar radii (Ca^{2+}, 0.99 Å; Cd^{2+}, 0.97 Å). Means *et al.* (Means, 1980) indicated that calmodulin (CaM) was located specifically in mitotic spindle and involved in the processes of chromosome movement through regulation and control of depolymerization and polymerization of microtubules (Cheung, 1980－1983). Also, Li *et al.* (Li, 1991) observed that CaM distributed in the nucleoli of the root tip cells of maize, suggesting that the nucleolar behavior might be regulated and controlled during the interphases. Cheng *et al.* (Cheng, 1991) found that there was more CaM in the cytoplasm, but the nuclei, especially nucleoli also contained CaM, when they studied the CaM distribution in the cultured tobacco cells using enzymelabelled immunohistochemistry.

From what we indicated above, we suggest that the toxic effects of Cd on nucleolus may result from low level of free Ca^{2+} in the cell, so the functions of CaM can not activated. But whether calcium has any protective effect against cadmium poisoning in nucleoli of root tip cells of *Allium cepa* should remain to be explained.

References

Bazynski T. Wajda L. Krol M, Wolinska D, Knipa Z, Tukendorf A. Physiol Plant, 1980; 48:365
Bazzaz FA, Rolfe GL, Carlson RW. Physiol Plant, 1974; 32:373
Cheng ZH, Bai J, Shi DW, Dun Dy. Acta Agr Boreali Sinica, 1991; 6(3):76
Chueng WY. Calcium and cell function. London, New York:Academic Press, 1980－1983
Ernst W. Bern Dtsch Bot Ges, 1972; 85:295
Fiskesjo G. Mutat Res, 1988; 197:243
Hampp R, Beulich K, Ziegler H. Z Pflanzenphysiol, 1976; 77:336
Huang CY, Bazzaz FA, Vanderhoef CN. Plant Physiol, 1974; 54:122
Lee KG, Cunningham BA, Paulsen GM, Liang GH, Moore RB. Physiol Plant, 1976; 36:4
Li JX, Sun DY. Chin J Cell Biol, 1991; 13(1):1
Liu DH, Jiang WS. Hereditas, 1991; 115:213
Liu DH, Jiang WS, Li MX. Acta Scientiae Circumstatiae, 1992; 12(4):339
Liu JS, Li ZS, Qu CX, J Mudanjiang Teachers College (Natural Sciences), 1988; 9(1－2):17
Means AR, Dedman JR. Nature, 1980; 285:73
Miller RJ, Bittell JE, Koeppe DE. Physiol Plant, 1973; 28:166
Risueno MC, Medina FL. Rev Biol Cell, 1986; 7:1
Takijema Y, Katsumi F, Tabezawa K. Soil Sci Plant Nutr, 1973; 19:178
Thornton I, Webb JS, Trace elements in soils and plants. In: Food chains and human nutrition (Ed. by Blaxter Sir Kenneth). Applied Science Publishers, 1979
Wang Y. General cell biology. Shanghai Scientific and Technic Publishing House, China, 1988:301
Weigel HJ, Jager HJJ. Plant Physiol, 1980; 65:480
Wen YY. Calcium and calmodulin. Beijing: Chemical Industry Publishing House, 1989:126

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