INFLUENCE OF HYDROXYUREA ON CELL DIVISIONS AND MICROTUBULAR CYTOSKELETON IN *ALLIUM CEPA* ROOT MERISTEM

H. Q. ZHANG¹, Y. Q. LI¹, M. KURAŚ², J. BEDNARA³, M. CRESTI¹

¹Environmental Biology Department, Siena University,
Via Mattioli 4, 53 100 Siena, Italy

²Plant Morphogenesis Department,
Banacha 2, 00-913 Warsaw University, Poland

³Plant Anatomy and Cytology Department,
Maria Curie-Skłodowska University,
Akademicka 19, 20-033 Lublin, Poland

ABSTRACT

In onion roots, hydroxyurea (HU) causes a gradual depression of mitotic activity which ceases after 24-36 hrs. The effect is reversible; divisions begin after several hours of recovery and after 12-14 hrs about 90% cells undergo mitosis.

Mitotic activity commences in the distal region of the apical meristem, and as a wave it spreads towards the apex.

In the roots treated with HU for a short time, the tubulin immunofluorescence method reveals normal arrays of microtubules (MTs). After 36 hrs of HU treatment there are only cortical and endocyttoplasmic MTs. In the recovering roots, preprophase bands (PB) mitotic spindles and phragmoplasts appear. Some PBs are split into two parallel rings. These abnormal PBs mostly occur in elongated cells. Apart from this, HU does not appear to have any significant influence on microtubular organization.

KEY WORDS: hydroxyurea, microtubules, mitotic activity.

INTRODUCTION

Hydroxyurea (HU) is an inhibitor of DNA synthesis and has little effect on the synthesis of RNA and proteins (Young et al. 1967, Rosenkrantz et al. 1966).

There is evidence that HU affects DNA synthesis by the depletion of cellular pools of deoxyribonucleoside triphosphates, because it substantially prevents the reduction of ribonucleotides to deoxyribonucleotides (Krakoff et al. 1968). Therefore, HU causes the prolongation of the G1 phase of the mitotic cycle and the depreciation of cell divisions.

Microtubular cytoskeleton is involved in the control and regulation of cell structure and cell cycle progression. Cytoskeletal tubulin can be easily demonstrated by immunofluorescence technique (Wick et al. 1981). The visualized microtubular skeleton occurs in typical arrays in the interphase and mitotic cells.

Numerous experiments have shown that drugs and growth substances influence the behaviour and arrays of microtubular cytoskeleton, mitotic spindles, phragmoplasts and interphase microtubules (Baluska and Barlow 1993; Gunning and Hardham, 1982; Roberts and Lloyd, 1985; Zhang et al. 1992).

We investigated the microtubular cytoskeleton in meristematic cells treated with HU and recovered after treatment. During its recovery the inhibition of DNA synthesis is easily reversible (Kihlman et al. 1966).

In recovering root meristems there is a sudden, substantial increase in mitotic activity. Therefore, the HU treatment may be applied to the cell cycle synchronization in the plant cell culture and in the root meristems (Sinclair 1965, Kihlman et al. 1966, Kuraś and Teleżyński, 1972).

MATERIAL AND METHODS

Onion (*Allium cepa* L.) bulbs with 3-5 cm roots grown in the dark at room temperature were placed in 0,3 mg/ml solution of hydroxyurea (HU Sigma) for 24 hrs, and subsequently transferred into 0,1 mg/ml for 12 hrs. Then, they were carefully rinsed and postincubated in water.

Root tips were collected; before incubation (control), during 36 hrs treatment with HU, and after 8, 10, 11, 12, 13, 14, 16, 18 hrs of postincubation.

For aceto orcein – squash preparations, root tips were fixed in a mixture of ethyl alcohol and acetic acid in the ratio of 3:1. For Feulgen reaction preparations, the root tips were placed for 24 hrs in CrAF (chromoacetic formaldehyde 0,5-1-20%). The pieces were then washed, dehydrated in ethanol, then through the xylol embedded in paraffin wax and cut into 7 µm longitudinal sections.

For immunofluorescence microscopy, the apical parts of roots were placed for 2 hours with 4% paraformaldehyde in PIPES

Fig. 1. Aceto-orcein stained preparations of the onion root tips

A – control – about 12% of mitotic nuclei

B – 36 h of hydroxyurea treatment – all mitotic activity inhibited

C – 14 h of recovery after HU treatment – very many nuclei in the mitotic stage.

Fig. 2. Mitotic activity in apical meristem of *Allium* roots; control, incubation in HU, recovery during postincubation.

buffer 0.05 M, pH 7.0 at 22°C. Following rinsing, the median free hand sections of roots were digested 30 min. with 0.5% cellulase and transferred to 1% Triton X-100 in PIPES buffer for 15 min. Following a 30 min rinse in buffer the sections were squashed on polylysine coated slides. The preparations

were incubated with mouse monoclonal antitubulin antibody (anti α tubulin Sigma) diluted 1:200 in PBS for 60 min at 37°C and FITC Goat Anti Mouse secondary antibody diluted 1:150 in PBS. Finally the preparations were stained with DAPI 1 μ g/ml and embedded in Cityfluor glycerol mixture.

Fig. 3. Mitotic activity in the apical part of onion root; A – control, B – 36h in HU, C – 10 h recovery after treatment, D – 14 h after treatment – shown maximal activity.

Fig. 4. The length of the cortical cells in the root apex in control and experimental roots.

The observations were made using Optiphot 2 Nikon microscope with epifluorescence and standard excitation and barrier filters.

RESULTS

Cell division figures were examined on squashed preparations of root meristems of control, treated with hydroxyurea and after the recovery period when the inhibitor was washed out from the tissue.

The average mitotic index of the control root meristem was about 12% (Figs 1A, 2). Mitotic activity gradually decreases in an onion root meristem treated with HU. After 36 hrs there is no mitotic cells (Figs 1B, 3B). This activity occurs again when the treated bulbs are transferred into water. After 8 hrs of recovery, the first mitoses appear in the distal region of the apical meristem. Then, after 10 hours, the number of mitoses in this region is comparable to that in a corresponding region of the control roots (Fig. 3A, 3C). The cells of the middle and of the apical region of the meristem are activated later, after 11 and 12 hrs of postincubation. After 14 hrs, mitotic activity is greatly increased in the whole meristem. The mitotic index exceeds 92 %. During the following postincubation period, mitotic activity gradually decreases to the normal level. In roots treated with hydroxyurea for 36 hrs, the cell divisions are blocked (Figs 1B, 2F, 3B). When the drug-treated roots were rinsed and allowed to recover for several hours, there was a progressive increase of mitotic activity. The mitotic index was about 6% in 10 hours of recovery, 11,2% in 12hrs, 92,7% in

14 hrs and after 16 hrs 18% of the meristem cells undergo mitosis. In HU treated and recovery meristems the length of cells are higher in comparison with control (Fig. 4) but the microtubule configurations is normal (Figs 5-6). There are cortical and endoplasmic microtubules in interphase stages, as well as the preprophase bands, mitotic spindles and phragmoplasts in the mitosis. In comparison with control, in recovery time the cells of meristems are longer, (Fig. 4). The tubulin cytoskeleton looks normal except the elongated shape of mitotic spindles and the double preprophase bands in some cells (Fig. 6).

DISCUSSION

Roots of *Allium cepa* bulbs were treated with hydroxyurea (HU) solution and fixed after different periods of treatment and recovery when HU was washed out. HU, a DNA synthesis inhibitor, prolongs the G1 phase of the mitotic cycle.

Mitotic activity disappears entirely after 24-36 hrs, but appears again when the roots recover in water. Within 8-11 hrs of recovery, the mitoses are originally concentrated in distal region of the meristem, but afterwards they gradually spread in apical direction. After 14 hrs of recovery, more than 90% cells undergoing mitosis.

We assume that the processes which prepare mitosis occur during the time of inhibition. The processes in the distal part of the meristem are apparently more advanced than those near the apex. The first mitoses appear after 8-10 and 12 hours respectively.

Fig. 5. Immunofluorescence of tubuline in meristematic cell of the *Allium* roots (x800)

A, B – endoplasmic and cortical microtubules

C-E – preprophase band formation

F-H – mitotic spindle

I – phragmoplast at telophase

J – partially desintegrated phragmoplast in late telophase

K – microtubules in posttelophase cells.

At first during HU incubation, the roots elongate almost normally. The non dividing meristem cells, particularly in the distal meristematic region, become noticeably longer than in control roots. In these elongated cells, wide or double preprophase bends are often set up. We assume that in elongating meristematic cells a wide area of cell membrane becomes adopted to anchorage of the preprophase bands. Consequently, twin preprophase bands may be formed. There is no evidence that both bands are simultaneously active in the formation of cell walls.

We have not observed any obvious abnormal microtubular structure in the interphase and mitotic cells, which were under the influence of HU. This compound does not seem to have a direct effect on the microtubular cytoskeleton.

ACKNOWLEDGEMENTS

We thank professor Bohdan Rodkiewicz for his helpful deal in preparing the manuscript.

Fig. 6. Immunofluorescence of tubulin in meristem cell of the *Allium* roots recovering after HU treatment. (x800)
A – elongated interphasal cell with the acentric nucleus
B-D – single and double preprophase bands
E – early prophase
F – microtubules of the metaphase spindle
G – late anaphase spindle
H – microtubules of the phragmoplast in the telophase cell.

LITERATURE CITED

- BALUSKA F., BARLOW P.I., 1993. The role of microtubular cytoskeleton in determining nuclear chromatin structure and passage of maize root cells through the cell cycle. *Eur. J. Cell Biol.* 61. 160-167.
- GUNNING B.E.S., HARDHAM A.R., 1982. Microtubules. *Annu. Rev. Plant Physiol.* 38. 651-698.
- KIHLMAN B.A., ERIKSSON T., ODMARK G., 1966. Effect of hydroxyurea on chromosomes, cell divisions and nucleic acid synthesis in *Vicia faba*. *Hereditas* 56. 386-397.
- KRAKOFF I.H., BROWN N.C., REICHARD P., 1968. Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Cancer Res.* 28. 1559-1565.
- KURAŚ M., TELEŻYŃSKI H., 1978. Influence of hydroxyurea on the course of germination and growth of rape (*Brassica napus* L.) seedlings. *Acta Soc. Bot. Pol.* 47(1-2). 131-141.
- ROBERTS J.N., LLOYD C.W., 1985. Ethylene – induced reorientations: mediation by helical arrays. *Planta* 164. 439-447.
- ROSENKRANZ H.S., GARRO A.J., LEVY J.A., CARR H.S., 1966. Studies with hydroxyurea. I. the reversible inhibition of bacterial DNA synthesis and the effect of hydroxyurea on the bactericidal action of streptomycin. *Biochim. Biophys. Acta* 114. 501-515.
- SINCLAIR W.K., 1965. Hydroxyurea: differential lethal effects on cultured mammalian cells during the cell cycle. *Science* 150. 1729-1731.
- WIC D.M., SEAGULL R.W., OSBORN M., WEBER K., GUNNING B.E.S., 1981. Immunofluorescence microscopy of organized microtubule arrays in structurally stabilized meristematic plant cells. *J. Cell Biol.* 89. 685-690.
- YOUNG CH.W., SCHOCHETMAN D.A., KARNOFSKY A., 1967. Hydroxyurea induced inhibition of deoxyribonucleotide synthesis studies in intact cells. *Cancer Res.* 27. 526-533.
- ZHANG D.H., WADSWORTH P., HEPLER P.K., 1992. Modulation of anaphase spindle microtubule structure in stamen hair cells of *Tradescantia* by calcium and related agents. *J. cell Sci.* 102. 79-89.

WPŁYW HYDROKSYMOCZNIKA NA PODZIAŁY I SZKIELET CYTOPLAZMATYCZNY
KOMÓREK MERYSTEMU KORZENIOWEGO *ALLIUM CEPA*

STRESZCZENIE

Hydroksymocznik powoduje stopniowy zanik podziałów w korzeniach cebuli, a po 24-36 godzinach całkowite ich zatrzymanie. Efekt jest odwracalny, po kilkunastu godzinach od przeniesienia do wody mitozy pojawiają się i po 12-14 godzinach ponad 90% komórek merystemu dzieli się. Mitozy zaczynają się w dyszałnej części merystemu i w postaci fali przesuwają się do szczytu.

Komórki z merystemów traktowanych hydroksymocznikiem mają normalny cytoszkielet tubulinowy, po 36 godzinach brak figur mitotycznych lecz zachowują się mikrotubule kortykalne i endoplazmatyczne. W korzeniach z których wypłukano hydroksymocznik pojawiają się zwykłe konfiguracje mikrotubul m.in. preprofazowe pierścienie, wrzeciono kariokinetyczne i fragmoplasty. Niektóre pierścienie preprofazowe widoczne w długich komórkach uległy rozerwaniu na dwa pasma.

SŁOWA KLUCZOWE: hydroksymocznik, mikrotubule, aktywność mitotyczna.