CHANGES IN THE NUMBER AND SIZE OF NUCLEOLI OF *CHARA VULGARIS* L. ANTHERIDIAL FILAMENT CELLS DURING THE PERIOD PRECEDING LIGHT-INDUCED RE-INITIATION OF CELL DIVISIONS FOLLOWING A MITODEPRESSIVE EFFECT OF DARKNESS

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ABSTRACT

The changes in number and size of nucleoli of *Chara vulgaris* antheridial filament cells were monitored with the use of Howell and Black's silver staining method. After a 3-day mitodepressive treatment with darkness the cells were exposed to light which reactivated mitotic activity after 18-20 hours. Eight-celled antheridial filaments were observed. In the period preceding light-induced re-initiation of mitoses a gradual reconstruction of the number and size of nucleoli characteristic of control, as well as their total area per nucleus appeared. The obtained results indicate that one of the important conditions for a cell to be able to divide is accumulation of nucleolus components characteristic of a given developmental stage and this controls nucleologenesis of the subsequent cell cycle.

KEY WORDS: *Chara vulgaris*, antheridial filaments, darkness, mitosis blockage, nucleoli.

INTRODUCTION

Antheridial filament cell cycle is vastly dependent on light factor (Maszewski 1977, 1981; Maszewski and Kwiatkowska 1982; Kwiatkowska and Maszewski 1980; Kwiatkowska et al. 1980; Kwiatkowska 1990; Kwiatkowska et al. 1992, 1995, 1999). The present experiments are a continuation of our previous work on ultrastructural changes in nucleoli brought about by dark-induced mitosis inhibition and during the time preceding their re-initiation caused by light (Kwiatkowska and Maszewski 1980).

Cytological, cytophotometrical and interferometrical studies have shown that all *Chara vulgaris* antheridial filament cells are inhibited by a prolonged darkness in the early G₂ phase, however, they vary in respect to their size and cytoplasm content (Maszewski and Kwiatkowska 1982). Smaller cells exhibit a marked deficiency of basic cytoplasm which is probably responsible for delayed re-entry into mitosis after light exposition. It was observed that only cells similar in size to those entering mitosis in control started to divide after 18-20 h of light exposition. The remaining ones were capable of dividing only after compensating cytoplasm deficiency (Maszewski and Kwiatkowska 1982). Due to this fact research on changes in the number and size of nucleoli during the period preceding light-induced re-commencement of mitoses took variations of cell size into consideration.

MATERIALS AND METHODS

Chara vulgaris antheridial filaments coming from antheridia of plants collected in natural environment were the ma-

terial of the experiment. The filaments were cultured in darkness for 3 days, aerated with an aerating pump-Skalar and then exposed to Flora light 4K lux for 6, 12, 18 hours.

Nucleoli were measured in light microscope following Howell and Black silver-staining (Howell and Black 1980). Apical parts of *Chara vulgaris* thallus were prefixed for 10 min in 2% glutaraldehyde with Sørensen's phosphoran buffer (pH 7.2) and later postfixed in ethanol acetic acid mixture (3:1 v/v) for 5 min at 4°C.

After fixing the material was rinsed in ethanol, gradually hydrated and rinsed in distilled water then put in a mixture consisting of: 2% gelly in 1% formic acid and 50% AgNO₃ (1:2 v/v). After silver staining at 60°C for 7 min the plants were thoroughly rinsed several times in distilled water and embedded in 5% sodium thiosulfate solution for 10 min. After rinsing in water the antheridia were isolated on gelatinized slides in 45% acetic acid. After removing cover slips the preparations were dehydrated, dried, and embedded in Canada balsam. Quantitative analyses for each time of light exposition and length of cells were carried out by counting and measuring nucleoli from 25 studied cells. The data were statistically analysed.

RESULTS AND DISCUSSION

The number of nucleoli in a nucleus

An average number of nucleoli in a nucleus decreases slightly in darkness as compared to control (Maszewski and Kwiatkowska 1984). This is probably due to diminution of the smallest nucleoli which become invisible in a light microscope. After 12-18 hours of light exposition (Fig. 1) the aver-

Fig. 1. Mean number of nucleoli per nucleus in cells with varied lengths just after darkness ("0" time) as well as after 6, 12 and 18 h of light exposition.

age number of nucleoli in a nucleus is similar to that in control L:D=14:10 (Kwiatkowska and Maszewski 1985b).

Changes in an average total volume of nucleoli in a nucleus

Figure 2 shows that immediately after exposition to darkness ("0" time) the nucleoli are smallest in all cell-size classes. Ultrastructural studies show that the decrease in nucleolus sizes in antheridial filaments grown in darkness for 3-5 days results from diminution of the area occupied by granular component, mainly in medium and big nucleoli, as small ones contain little of it also in control (Kwiatkowska and Maszewski 1980). After 60 min light exposition the first sign of ultrastructural changes in antheridial filament cell nucleus is the appearance of a small amount of granular component in nucleoli (Kwiatkowska and Maszewski 1980). Light microscope measurements after 6 h of light exposition show a distinctive increase in total nucleoli volume in all cell-size classes. In the biggest cells nucleoli reach maximum sizes, nearly 5 times higher than those at "0" time, after 18 hours. The smallest cells in which a slight elongation may be observed (with constant cell width), exhibit a lower increase in total nucleoli volume after 18 h of light exposition. This may be due to fast maturation and transport of ribosomal subunits out of a nucleoli in connection with supplementing cytoplasm deficit in these cells (Maszewski and Kwiatkowska 1982). Accumulation of argenophilic material in nucleoli in cells approaching division may be a preparation to next cell cycle commencement (Kwiatkowska and Maszewski 1985a, 1985b, 1985c).

Percentage of 5 classes of nucleolus sizes in different classes of cell lengths

Diagrams presented in Fig. 3 show that just after dark exposition the diameters of approximately 50-60% of nucleoli are between 0.20-0.43 μm and of 20-35% of nucleoli between 0.44-0.87 μm. The remaining 10-20% are the nucleoli with diameters 0.88-1.30 μm and 1.31-1.74 μm. Six hours after light exposition a marked decrease in the percentage of the smallest nucleoli and increase in the number of the remaining size classes appear. Less dynamic changes can be observed in

Fig. 2. Changes in average total volume of nucleoli in a nucleus preceding photoinduced re-initiation of mitoses.

Fig. 3. Percentage of 5 classes of nucleoli sizes in different classes of cell lengths according to light exposition duration.

the smallest cells. Only after 18 h of light in all classes of cell sizes nucleoli with a diameter more 1.74 μm appear. They are most numerous in the biggest cells, mainly due to a reduction in the number of nucleoli with diameters ranging from 0.20 to 0.43 μm .

The number of various-sized nucleoli expressed in absolute numbers in the time preceding light-induced re-initiation of mitoses as compared with preprophase cells in control

Data shown in Fig. 4 indicate that in plants exposed to 3-day darkness, followed by 18 h of light, in longest cells the percentage of nucleoli of different size classes was very similar to that observed in preprophase cells with a length equal to that of cells entering mitosis. This means that before light-induced re-initiation of mitoses there is a total reconstruction of nucleolus sizes in a nucleus. A conclusion can be drawn that one of the conditions of photoinduced re-initiation of mitotic divisions is the accumulation of nucleolus components characteristic of the given developmental stage of antheridial filaments. These components are accumulated for the next cell cycle and take part in nucleologenesis process conditioning its dynamics. The bigger the preprophase of nucleoli of the previous cycle, the faster the development of nucleoli in the next antheridial filament cycle in *Chara vulgaris* (Kwiatkowska and Maszewski 1985b). This result was thoroughly supported by experiments on 4 other *Chara* species: *C. fragilis* Desv., *C. contraria* Kütz., *C. aspera* Willd. i *C. tomentosa* L. (Maszewski 1994). It is probably a chain of

Fig. 4. The number of various-sized nucleoli expressed in absolute numbers in the time preceding light-induced re-initiation of mitoses as compared with preprophase cells in control.

changes occurring in subsequent cell cycles of antheridial filaments preceding spermiogenesis leading to morphogenetic reduction of cell sizes and blockage of mitotic divisions.

The fact that nucleolus components, dispersed during mitosis, find their way back to nucleoli recreated in daughter cells was proved, among others by immunocytochemical studies in light and electron microscopes (Medina et al. 1995; Schwab et al. 1998).

Slowed down accumulation of nucleolus components during light exposition observed in our studies in antheridial filament cells which, after darkness, were the smallest may be one of the reasons for their delayed maturity for mitosis. These cells divide in the second division wave, a few hours after the first one (Maszewski 1979).

It is also one of the reasons for lack of synchrony of mitotic divisions triggered by light after a mitodepressive effect of darkness in spite of the fact that darkness stops the nuclear cycle of antheridial filament cells at the same stage-middle G₂ phase (Kwiatkowska and Maszewski 1980; Kwiatkowska et al. 1980).

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ZMIANY LICZBY I WIELKOŚCI JĄDEREK
W KOMÓRKACH NICI SPERMATOGENICZNYCH *CHARA VULGARIS* L.
W OKRESIE POPRZEDZAJĄCYM PONOWNĄ INICJACJĘ PODZIAŁÓW KOMÓRKOWYCH
PO WPŁYWEM ŚWIATŁA PO MITODEPRESYJNYM DZIAŁANIU CIEMNOŚCI

STRESZCZENIE

Stosując technikę srebrzenia Howella i Blacka zbadano zmiany liczby i wielkości jąder w niciach spermatogenicznych *C. vulgaris* L. poddanych najpierw 3-dobowemu mitodepresyjnemu działaniu ciemności, a następnie eksponowanych na świetle, które reaktywuje aktywność mitotyczną po ok. 18-20 godz. Obserwacje dotyczą 8-komórkowego stadium rozwojowego nici spermatogenicznych. Stwierdzono, że w okresie poprzedzającym fotoindukcyjne wznowienie mitoz następuje stopniowa rekonstrukcja charakterystycznej dla kontroli liczby i wielkości jąder oraz ich sumarycznej wielkości przypadającej na jądro. Uzyskane wyniki wskazują, że jednym z istotnych warunków osiągnięcia przez komórkę zdolności do podziału jest nagromadzenie charakterystycznego dla danego stadium rozwoju, poziomu składników jąderkowych, który decyduje o przebiegu nukleogenezy następnego cyklu komórkowego.

SŁOWA KLUCZOWE: *Chara vulgaris*, nici spermatogeniczne, ciemność, blokowanie mitoz, jąderka.