PCC-LIKE INDUCTION OF MITOSIS IN *CHARA VULGARIS* ANTHERIDIA INITIATING DIFFERENTIATION OF SPERMATOZOIDS IN THE DARKNESS

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ABSTRACT

The objects of this research were the antheridial filament cells of *Chara vulgaris* which after 5-day mitodepressing treatment of the darkness reactivated mitosis (without light stimulation) in the result of the physiological state evoking the initiation of spermiogenesis in the antheridium. The course of these divisions was observed in relation to control cells cultivated in the natural photoperiod L:D = 14:10. It was shown that the passage of dark-arrested cells from early G₂ (VII stage) to the early prophase in the cells linked to the base of filaments by plasmodesmata with spermatids initiating spermiogenesis, resembles the prematurely condensed chromosomes (PCC). In this way a by-pass of the cells in the stage VII to early prophase occurs without reconstruction of granular components of nucleoli and with condensed stage of mitochondria characterising the dark-arrested cells. In opposition to this phenomenon in the control cell cycle and in light-induced reactivation of mitosis in the dark-arrested cells, initiation of the prophase follows the series of changes of the nuclear ultrastructure (VIII stage – decondensation of chromatin, IX and X stages nuclei with reticulate chromatin).

At the early prophase, irrespective of the physiological conditions, the electron-transparent space appears between nuclear envelope and the chromatin as the effect of solubilisation of lamins. The fine fibrils pass this space and link chromatin to the inner nuclear membrane.

KEY WORDS: *Chara vulgaris*, antheridia, PCC-like mitosis, spermiogenesis, darkness.

INTRODUCTION

Antheridial filaments of the alga *Chara vulgaris* represent a suitable material for studies on the interdependence of nuclear structure and function during mitotic cycle. The length of interphase cells which divide synchronously in a filament after reaching definite mitotic sizes during successive cycles may be accepted as a simple and precise indicator of a given stage of interphase. The type of nuclear cycle in haploidal antheridial filaments was found to be S+G₂+M (Olszewska and Godlewski 1972; Kwiatkowska and Maszewski 1978).

Using both, the cell length criterion and ³H thymidine labelling analysis for recognizing particular phases of the cell cycle, we have described ten types (6 in S-phase and 4 in G₂-phase) of nuclear ultrastructure during interphase.

They were manifested mainly by modifications of the arrangement and content of condensed chromatin (Kwiatkowska and Maszewski 1978). In the later stages (types IX and X) a progressively increased process of chromatin condensation precedes the arrangement of mitotic chromosomes.

The course of the cell cycle in antheridial filaments of *Chara vulgaris* is greatly influenced by changes in photoperiodic conditions. In plants grown totally in the darkness for 3-5 days, complete cessation of cell divisions was shown in all antheridial filament generations (2-4-8-16-32-celled filaments). The arrest of the mitotic cycle was found to be re-

versible; all stages of antheridial filaments resumed cell division within some 18 hrs, after backshift to light (Maszewski 1977).

Antheridial filaments in which mitotic divisions ceased in response to a 5-day dark treatment, displayed a considerable homogeneity of nuclear morphology and ultrastructure. All nuclei, irrespective of cell length, show large and variable sized electron dense masses of condensed chromatin. Substantial amounts of condensed chromatin are localised at nuclear periphery in attachment to the envelope and in close association with nucleoli (Kwiatkowska et al. 1980). The comparison of nuclear organization between cells blocked by extended period of dark treatment and control cells during successive phases of the cell cycle has indicated that the former population exhibits evident convergence of morphological features merely to the nuclei of type VII from early G₂ phase and containing 2C DNA (Kwiatkowska and Maszewski 1980; Maszewski and Kwiatkowska 1982). Both compared populations show also similar contents of condensed chromatin. However two evident differences exist between control cells of type VII and dark-treated cells; that is: 1) the lack of granular components in the nucleoli together a marked reduction of nucleolar sizes and 2) very condensed structure of mitochondria (Kwiatkowska et al. 1980; Kwiatkowska and Maszewski 1980).

Results of the ultrastructural study of the light-induced resumption of mitotic activity support the conclusion that dark inhibited cells are arrested in the only one and definite stage of the G_2 phase. This is evidenced by the appearance of nuclear structures which correspond with successive types of the control G_2 phase nuclei between type VII and mitosis, in the period preceding photoreactivation of mitosis (Kwiatkowska and Maszewski 1980). Ultrastructural changes occur synchronously in all cells of a given filament; they are, however, not synchronized within the population of filaments composing a single antheridium. This is clear in view of the fact that merely some 20% of filaments enter mitosis after 18 hours of exposure to light conditions, owing to a dissociation of the nuclear cycle and the cell growth cycle in antheridial filament during the 5-day dark treatment (Maszewski and Kwiatkowska 1982). In the period preceding photoinduced resumption of mitotic divisions in antheridial filaments a renewed arrangement of granular component of nucleoli takes place: its amount increases gradually in parallel with the nucleolar growth. The above process was found to be the first detectable change of nuclear structure during the reactivation of the dark-inhibited cells (Kwiatkowska and Maszewski 1980).

Earlier studies (Kwiatkowska and Maszewski 1987) have demonstrated that the cell cycle blocking in the darkness takes place only in the antheridia in which spermatids have not appeared yet or their number in the antheridium is very small. In these antheridia the process of the spermatozoid differentiation is not initiated, and dark-treatment above 7 days causes the degeneration of the antheridial filaments. The antheridia with greater number of filaments at the stage of spermatids are capable of initiating spermiogenesis – which is insensitive to the light conditions. In the control this process takes place within an antheridium simultaneously in all fila-

ments. In darkness, however, the differentiation of spermatids begins in spite of the fact that the synchronization of development of antheridial filaments has not been established yet. Apart from the filament differentiating cells there are antheridial filaments which, due to cell cycle blocking in early G_2 phase, have not attained the stage of spermatids with 1C DNA level. The fate of these antheridial filaments may be as follows (Kwiatkowska and Maszewski 1987):

- a) the most frequently occurring phenomenon is the initiation of the mitotic divisions in the cell cycle of arrested filaments. In some cases these mitoses can take place simultaneously in many filaments.
- b) another situation can be detected in the antheridia in which the retarded filaments constitute a small portion of the population and the filaments at the stage of spermatids predominate. In delayed filaments one can observe incorrect chromatin condensation like in apoptosis (Oberhammer et al., 1994). The development of these filaments is then stopped and they do not initiate the differentiation.
- c) the most rarely existing possibility is the initiation of spermatozoid differentiation in the antheridial filament cells containing 2C DNA level, which were blocked at the G_2 phase and during the initiation of spermiogenesis they did not manage to divide together with other delayed filaments.

The chief aim of our observations was to analyse the course of mitosis in the filaments in which the arrest caused by darkness was broken (without light) in those antheridia which are mature enough to be able to enter spermiogenesis (point (a) above). These divisions have been observed only in the filaments adjoining those ones in which early symptoms of spermatid differentiation have been visible (Figure 1).

Fig. 1. Schematic illustration of plasmodesmatal connections in *Chara vulgaris* L. within capitular cell – antheridial filaments complex: dark-arrested antheridial filament in stage G_2 (1), in stage of late telophase (2), and during initiation of spermiogenesis (3). c – capitular cell; p – plugged plasmodesmata; o – open plasmodesmata

MATERIALS AND METHODS

Apical parts of thalli carrying antheridia from a genetic line of oospore-grown *Chara vulgaris* L. cultured axenically in Forsberg's mineral medium were used for testing. Control material was cultivated in L:D = 14:10 photoperiod. The block of mitotic divisions in antheridial filaments was produced by a 5-day aerated culture in darkness.

Thalli were fixed for 2 hours at room temperature in 3% glutaraldehyde in 0.0125 M phosphate buffer, pH 7.3. For light microscopical estimation of a given stage of development, antheridial filaments were detached from shield cells by squeezing the antheridia gently in a drop of agar. Selected agar dice with antheridial filaments were posfixed in veronal buffered (0.0125 M; pH 7.3) 1% OsO₄ solution dehydrated in an ethanol series and embedded in Epon 812 in flat plastic moulds. The ultrathin sections were made from the antheridia with 32- and 64-celled antheridial filaments selected under the light microscope. They were double stained in uranyl acetate followed by Reynold's lead citrate.

The structure of nuclei was analysed on micrographs from longitudinal sections of the filaments containing equatorial profiles.

RESULTS

The variability of the ultrastructure of nuclei during interphase of antheridial filament cells is in strong opposition to the monotonous ultrastructure of nuclei blocked at the early G₂ phase after 3-5 days of darkness treatment of plants (Kwiatkowska et al., 1980). The photoinduced release of cells from their arrest point generates the renewal of rearrangement of nuclear ultrastructure. These changes are a repetition of a process occurring between early G₂ phase (type VII) and mitosis in the control (Kwiatkowska and Maszewski, 1980).

In the case of induction of mitosis without the participation of light by physiological state releasing the initiation of spermiogenesis in spermatids, the course of ultrastructural changes in the nuclei is entirely different (Figure 2). The crucial point is a by-pass of nuclear structure type VII to the prophase, with a complete pass over intermediate processes. The regeneration of granular components of nucleoli and augmentation of their sizes to the level of control preprophase are not observed either (Figure 5).

Moreover all the mitochondria have a condensed structure like those in dark-arrested cells (Figures 3-7). However, one of the symptoms of prophase is visible: between inner nuclear membrane and chromatin the light space appears. In the same places fine chromatin fibrils pass this space and are linked to the inner nuclear membrane (Figures 4 and 5). Similar situation can be seen in a normal early prophase of antheridial filament cells (Kwiatkowska and Maszewski 1978).

In antheridia in which the initiation of spermiogenesis and mitosis takes place successive phases of mitosis can be also observed (Figures 6 and 7). They differ from control cells in the structure of mitochondria, which have permanently a very condensed structure of matrix (Figures 4-7).

The induction of divisions in antheridial filament cells correlated with a period of initiation of spermatid differentiation in the adjoining antheridial filaments does not always lead to the correct mitoses (Figure 7). However in many cases normal division of chromosomes takes place, and the descendant cells achieve 1C DNA level (Kwiatkowska and Maszewski 1987).

DISCUSSION

The induction of divisions in antheridial filament cells of *Chara vulgaris* correlated with a period of initiation of spermatid differentiation in the adjoining filaments enables the return to the synchronization of spermatogenesis in antheridium

Fig. 2. A – Changes of the ultrastructure of antheridial filament nuclei during G₂ and prophase in the control. B – The structure of dark-arrested cell nuclei at the early G₂ and early and late prophase in conditions causing initiation of spermiogenesis in darkness. (Control stages VII to early prophase were detailed documented on the photograms by Kwiatkowska and Maszewski (1978).

Fig. 3. Dark-arrested cell of antheridial filament. Nucleus structure like in VII stage. Mitochondria (m) in very compact state. Plasmodesmata (p) filled with light cytoplasm.

disturbed by the mitodepressing effects of darkness. These effects inhibit the last mitosis of some antheridial filaments. Retarded filaments can in this way achieve the stage of spermatids.

Entering immediately of the early prophase by the dark-blocked cells being in the early G_2 phase is suggested image of the nucleus, which has the structure of chromatin and nucleolus similar to the dark-arrested cells, being at the same time in the stage of disappearance of nuclear lamina interposed between the chromatin and the inner nuclear membrane (Aebi et al. 1986).

The lamins, which belong to two general classes (type A, and type B lamins) are able to bind specifically to chromatin fragments in vitro (Georgatos 1994). On this basis, it has been suggested that the nuclear lamins physically interact with a proteinaceous component of chromatin. On the other hand, the lamins are thought to interact with the inner nuclear membrane by binding to integral membrane proteins such as the lamina associated polypeptides and p58-lamin B receptor (Worman et al. 1988). It has been shown that integral membrane proteins of the nuclear envelope may be directly associated with chromatin as well (Georgatos 1994). The mitotic modification, principally by $p34^{cdc2}$ -mediated phosphorylation of lamins, results in an easily observable disassembly of nuclear lamina in the prophase. Fine fibrils which link chromatin to inner membrane of nuclear envelope after solubilization of lamins in the early prophase of antheridial filament cells seem to correspond with the results of precited authors. Similar phenomena can be seen in other cells of plants and animals (Robbin and Gontas 1964; Pickett-Heaps and Northcote 1966; Bajer and Molé-Bajer 1969; Pickett-Heaps 1969;

Comings and Okada 1970; Matsui et al. 1972; Stack et al. 1977). The present observations suggest that this process in the early G_2 arrested antheridial filament cells may be induced by spermatids with a by-pass of the normal changes in the structure of chromatin and nucleolus during middle and late G_2 phases. This phenomenon seems to resemble the results of experiments with fusion between interphase and mitotic protoplasts inducing premature chromosome condensation (PCC) (Robins and Gontas 1964; Szabados and Dudits 1980; Sunkara et al. 1980). This study clearly suggests that factors for PCC induction are present in the cytoplasm of the mitotic cells (Sunkara et al. 1980).

On the other hand incubation of demembrated sperm chromatin in cytoplasm extracted from unfertilized *Xenopus laevis* eggs results in nuclear envelope assembly, chromosome decondensation and sperm pronuclear formation (Lohka and Maller 1985).

In antheridial filaments there is no direct contact of spermatids initiating spermiogenesis and G_2 phase cells arrested in darkness. However, the basal cells of the antheridial filaments growing from the same capitulum are connected by the large plugged plasmodesmata filled with osmophilic substance (Kwiatkowska and Maszewski 1985, 1986) which probably enable the penetration of a factor inducing mitosis (Figure 1). The results of our studies on the intercellular connection within antheridia of *Chara vulgaris* lead to the assumption that the plugged plasmodesmata enable the preservation of the individual course of development of the cells they connect, whereas the plasmodesmata filled with the transparent cytoplasm (Figure 5) entail the complete subordination of the cell development to the whole programme realized by the

Figs 4 and 5. Dark-arrested cells in early prophase with light space (ls) between the chromatin and nuclear envelope. m – mitochondria; n – nucleolus; p – plasmodesmata; f – fibrils of chromatin linked to nuclear envelope

Figs 6 and 7. Late prophase and telophase in the darkness from antheridia with filaments at early spermiogenesis. m – condensed mitochondria; cp – cell plate. Fig. 7, on the right – incorrect division.

symplast (Kwiatkowska and Maszewski 1985, 1986). Therefore, the existence of such complexes of the capitular cells and the antheridial filaments in which some filaments begin the spermatid differentiation, others initiate mitosis (Fig. 1) is possible until the plasmodesmata plugged with the osmophilic substance enable the specific response of the cell, depending on its competence.

Opening of plasmodesmata (Kwiatkowska and Maszewski 1985, 1986) leads to the free exchange of the regulating substances in the whole symplast and to the infiltration of the compounds produced by normal spermatids which have initiated spermiogenesis to the cells with double DNA content, inducing them to the differentiation into abnormally big spermatozooids (Kwiatkowska and Maszewski 1987).

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INDUKCJA MITOZY PRZYPOMINAJĄCA PCC W ANTERYDIACH *CHARA VULGARIS* INICJUJĄCYCH DYFERENCJACJĘ SPERMATOZOIDÓW W CIEMNOŚCI

STRESZCZENIE

Obiektem tych badań były komórki nici spermatogenicznych *Chara vulgaris*, które po 5-dniowym mitodepresyjnym działaniu ciemności wznowiały mitozy (bez stymulacji światłem) wskutek stanu fizjologicznego wywołującego inicjację spermiogenezy w anterydium. Przebieg tych podziałów był porównywany z podziałem kontrolnych komórek anterydiów hodowanych w naturalnym fotoperiodzie L:D=14:10. Wykazano, że przejście zablokowanych ciemnością komórek z wczesnej fazy G₂ (VII stadium) do wczesnej profazy w komórkach połączonych u podstawy nici plazmodesmami ze spermatydami inicjującymi spermiogenezę, przypomina przedwczesną kondensację chromosomów (PCC). Następuje przeskoczenie ze stadium VII do wczesnej profazy, bez rekonstrukcji granularnego składnika w jąderkach, z zachowaniem mitochondriów w stadium kondensacji charakterystycznym dla zablokowanych ciemnością komórek. W przeciwieństwie do tego zjawiska w komórkach odbywających cykl komórkowy w warunkach kontrolnych, a także w indukowanych światłem mitozach zablokowanych ciemnością komórek, inicjacja profazy następuje po serii zmian w strukturze jądra (VIII stadium – dekondensacji chromatyny, IX i X stadia jądra z siatkową chromatyną). We wczesnej profazie, niezależnie od warunków, pojawia się przezroczysta dla elektronów strefa pomiędzy otoczką jądrową a chromatyną w efekcie solubilizacji lamin. Strefę tę przenikają cienkie fibryle, które łączą chromatynę z wewnętrzną błoną otoczki jądrowej.

SŁOWA KLUCZOWE: *Chara vulgaris*, anteridia, mitoza podobna do PCC, spermiogeneza, ciemność.