The role of plant growth substances in the regulation of the cell cycle in antheridial filaments of *Chara vulgaris* L.

I. Effect of gibberellic acid on some processes in the course of the cell cycle

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Abstract

The effect of gibberellic acid (10⁻⁴ M) on the incorporation of 8-¹⁴C adenine, ³H phenylalanine, the dimensions of mitotic cells and the durations of particular stages in the cell cycle were studied in synchronously dividing cells of the antheridial filaments in *Chara vulgaris* L. during successive periods of growth and differentiation.

GA₃ strongly stimulates the uptake of both labeled precursors in the course of a whole interphase and in all generations of the antheridial filaments; approximatively in proportion to the intensity of the process in the control. The gibberellin causes a slight increment in cell dimensions and strongly reduces the cell cycle durations: the S, G₂, and M to a similar degree. The earlier is the generation of the antheridial filament, the more pronounced is the influence of the plant growth substance.

Since the gibberellin stimulated the course of all examined processes, the present study did not reveal any stage of interphase to be especially sensitive to GA₃. The results suggest to interpret the effect of GA₃ as an unspecific stimulator of metabolism in cells of the antheridial filaments of *Chara vulgaris* L.

INTRODUCTION

Using mainly the techniques of biochemistry numerous investigators have shown the stimulating effect of gibberellins on a variety of physiological processes. Relatively little work was done by means of the cytophysiological analysis which allows the characterization of the influence of these substances on the metabolism of particular cells. Complex studies

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on the effect of gibberellin on the course of different processes in the successive stages of cell cycle are lacking.

The purpose of the present investigation was to study the effect of gibberellic acid (GA₃) on the dimensions of cells, the duration of particular stages in the cell cycle and the intensity of incorporation of labeled precursors into nucleic acids and proteins in the course of interphase in the antheridial filaments of Chara vulgaris L. These cells provide an especially suitable material in the studies of this kind, since they exhibit a high degree of spontaneous synchrony within a filament and their size is correlated with the period of interphase (Olszewska and Godlewski, 1972). Moreover, this material enables the analysis of the influence of plant growth regulator on cells of different metabolic activity, since the successive divisions are accompanied by the process of differentiation leading to the formation of spermatozoids. Its manifestation is the gradual reduction of both the cell growth and cell cycle duration, the decrease of the intensity of incorporation of labeled phenylalanine and 8-¹⁴C adenine into RNA, and also the reduction of ³H actinomycin D (³H AMD) binding capacity into the nuclear DNA (Olszewska and Godlewski, 1972; Godlewski and Olszewska, 1973; Olszewska, 1974).

The finding of the "gibberellin-like" substances in the thallus of Chara (Murakami, 1966) accounts for the choice of this material as the object of the present study.

MATERIALS AND METHODS

Plants for experiments were obtained from the pond in the village Pełczyska (Łódź district), the place which also was the source of water used both for the cultivation of the material and for the preparation of the solutions. This water, pH 7.45, was found to contain about 0.3 per cent of salts (Godlewski, 1973).

The apical parts of the thallus including nodes carrying antheridia were placed into the solution of gibberellic acid (10⁻⁴ M) for 24 hours and then kept with ³H thymidine (60 μCi/ml, 5 mCi/mM), or 8-¹⁴C adenine (6.25 μCi/ml, 54 mCi/mM), or ³H phenylalanine (20 μCi/ml, 1.53 mCi/mM) for 2 hours. In order to determine the duration of the cell cycle after the incubation with labeled thymidine, the material was postincubated for 15, 20, 25, 30, 35, and 40 hours. The plants were fixed in ethanol — acetic acid mixture (3 : 1). Isolated antheridia were squeezed out onto slides, covered with Ilford L4 liquid nuclear emulsion and exposed: for 8 months — after incubation with radioactive thymidine, for 2 days — preparations labeled with 8-¹⁴C adenine, and for 7 days — those after incubation with ³H phenylalanine. Autoradiograms were stained with Unna's mixture.

The effect of GA₃ on the incorporation of 8-¹⁴C adenine and ³H phenylalanine was estimated for cells in the five successive stages of their deve-
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Development, i.e. in 2-, 4-, 8-, 16-, and 32-celled filaments. On the basis of cell-dimensions five consecutive steps of interphase were distinguished for all examined generations. In the case of 8-14C adenine the radioactivity of nuclei was determined while after incubation with 3H phenylalanine nuclear and cytoplasmic incorporations were studied. Number of grains or traces (when 3H or 14C were used, respectively) was calculated by means of an eyepiece squared micrometer.

The mean values illustrating the intensity of incorporation for each stage of interphase were obtained from the analysis of 50 to 100 cells in 4 to 5 antheridia.

The duration of the cell cycle was estimated using the autoradiographic method with labeled thymidine. In the antheridial filaments of Chara vulgaris the cell cycle begins with DNA synthesis as early as the late telophase of the preceding generation, which also is the period of the most intense incorporation of thymidine. The passage of time from that moment till the appearance of strongly labeled telophases assignes the duration of the cell cycle (Godlewski and Olzewska, 1973). In order to determine the mean value of the cell cycle, radioactivity of nuclei after successive intervals of postincubation was calculated according to the four-step empiric scale.

Determination of S phase duration was based on the calculation of the percentage of cells incorporating labeled thymidine in the analysed stage of the filament (Monesi, 1969). The result is referred to the known duration of the cell cycle as the product of both these values. For this purpose preparations made from the material fixed immediately after incubation were examined. Since the estimated duration of S phase is prolonged by the period of incubation with radioactive thymidine, it was diminished by the subtraction of a value equal to the ratio of 2-h incubation period to the duration of the whole cell cycle.

RESULTS

Cell length measurements of the antheridial filaments at successive stages of their development showed that GA3 does not change or causes the increment of the dimensions of the mitotic cells. In a few experimental repetitions the length of miotic cells in plants incubated with the gibberellin was over a dozen per cent greater as compared with the control material.

Effect of GA3 on the duration of the cell cycle

The duration of the cell cycle in the successive generations of the antheridial filaments in the control material is shorter and shorter, in
agreement with previous results of Godlewski and Olszewskaya (1973). GA₃ accelerates the course of the cell cycle in all analysed stages of the antheridal filaments, the reaction of cells at earlier stages being the most pronounced (Fig. 1). The mean duration of the cell cycle in the

![Graph showing cell cycle duration](image)

Fig. 1. Cell cycle durations in the 2-, 4-, 8-, 16-, and 32-celled antheridal filaments in the control material (K) and after incubation with GA₃

2-celled filaments was reduced of about 13 h., i.e. 32 per cent, in 4-celled filaments of about 8 h., i.e. 22 per cent, in 8-celled — of about 5 h., i.e. 15 per cent, and at later stages of the development — that is in 16-, and 32-celled filaments — of about 1.5 and 2.5 h., i.e. 6 and 10 per cent, respectively.

The method used enables a determination of S phase duration, and after subtraction of that period from the duration of the cell cycle it is possible to establish the rest of the cycle, i.e. G₂ phase and mitosis. GA₃ reduces the duration of both periods of the cell cycle (Fig. 2). At early stages of the development, which are characterized by the longest duration of G₂ phase, the reduction of both G₂ and mitosis is greater, as compared with the reduction of S phase. In the 2-celled filaments the S phase and G₂ + M phases were reduced of 3.2 and 8.7 h., i.e. 38 and 51 per cent,
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respectively; in 4-celled of about 1.8 and 6.2 h., i.e. 13 and 38 per cent. In the filaments being at later stages of their development, reduction of the duration of total cell cycle was similar, and in the last generation

Fig. 2. Duration of S phase and G2 + mitosis (M) in the 2-, 4-, 8-, 16- and 32-celled filaments in the control (K) and after incubation with GA3

which takes a course throughout all periods of the cycle and which has the shortest G2 phase — the 32-celled filaments — the only period reduced was the S phase.

Effect of GA3 on the incorporation of 8-14C adenine

GA3 stimulates the incorporation of 8-14C adenine into nuclei of all examined stages of the antheridial filaments (Fig. 3). The stimulation of the uptake of 14C-labeled purine is similar in all periods of the develop-

Fig. 3. Incorporation of 8-14C adenine into nuclei at interphase of the 2-, 4-, 8-, 16- and 32-celled filaments in the control (K) and after incubation with GA3
ment and during a whole interphase. It seems, however, that the intensity of incorporation of the precursor into posttelophase cells (early S phase) of 8-, 16-, and 32-celled filaments is slightly greater than at earlier stages of the development, i.e. in the 2-, and 4-celled filaments. The uptake of 8-\(^{14}\)C adenine in \(G_2\) is stimulated mostly into 8-celled filaments, although it is there only a little stronger than into cells of other generations of the antheridal filaments staying at the same period of interphase.

**Effect of \(GA_3\) on the incorporation of \(^3\)H phenylalanine**

\(GA_3\) stimulates strongly the incorporation of labeled phenylalanine into the cells of all studied generations of the antheridal filaments (Fig. 4). The higher is the level of incorporation of the precursor into cells in the control material, the more pronounced is the increment of the uptake of labeled amino acid into the nucleus and cytoplasm under the influence of the gibberellin. Thus, the intensity of incorporation of labeled phenylalanine after the incubation with \(GA_3\) occurs most strongly into cells of the 2-celled filaments; it decreases in the successive generations of the filaments, but the ratio of incorporated radioactive amino acid in the

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Fig. 4. Incorporation of \(^3\)H phenylalanine into nuclei (n) and cytoplasm (c) at interphase of the 2-, 4-, 8-, 16-, and 32-celled filaments in the control (K) and after incubation with \(GA_3\).
control conditions and under the influence of GA$_3$ is approximately still at the same level. Since the curve illustrating the intensity of the uptake does not show a tendency to decline in the course of interphase, as it occurs in the control material, the gibberellin stimulates a little more the incorporation of the precursor in G$_2$ phase (which is especially marked in the cells of 4-, 8-, and 16-celled filaments).

**DISCUSSION**

Since a "gibberellin-like" substance was found in the thallus of Chara (Murakami, 1966), one can suppose, that like in the higher plants gibberellins play a role in the regulation of metabolic processes determining growth of these algae. Gibberellic acid in the concentration of $10^{-4}$ M, used for the incubation of plants, exhibits the stimulating effect on all examined processes during the cell cycle in the antheridial filaments of Chara vulgaris. It stimulates the incorporation of precursors of nucleic acids and proteins and promotes cells having their dimensions near to that of the control's or larger to enter mitosis.

The stimulation of 8-$^{14}$C adenine incorporation into nucleic acids was ascertained in all studied generations and it was found to be similar in the course of a whole interphase with only small deviations. The enhancement of incorporation of labeled precursor during S phase indicates the stimulating effect of GA$_3$ on both DNA and RNA syntheses while that during G$_2$ phase, which is the main period of RNA synthesis, denotes the stimulation of transcription. Induction of RNA synthesis during incubation in the physiological solutions of GA$_3$ was found in different material (Fletcher and Osborne, 1966; Pinfield and Stobart, 1969; Rejowski and Kulka, 1970). Sometimes it may be capable of the increment in number of particular RNA fractions, especially mRNA (Chandra and Duynastee, 1968; Korableva and Ladyrhen-skaya, 1971; Zwar and Jacobsen, 1972; Durant, 1972; Kleczkowski, pers. comm.).

The stimulation of RNA synthesis enables the connection of the effect of gibberellin with the process regulating transcription: DNA template activity and RNA polymerase activity. The experiments on the binding of labeled actinomycin D into nuclei of the antheridial filaments of Chara vulgaris showed that GA$_3$ considerably increases the number of sites capable of binding the antibiotic, especially in G$_2$, and they suggest that the plant hormone may act directly or indirectly at the transcription level (Olszewska and Godlewski, unpubl.). This effect may consist in the prolonged action of genes responsible for RNA synthesis, or else may depend on the activation of new genes.
Experiments performed on the isolated chromatin which showed the impairment of linkage between DNA and histones and the increment of hyperchromicity during the thermal denaturation of DNA isolated from corn germs in the presence of GA₃ also indicates the possibility of action of the growth regulator at the transcription level (Fellenberg, 1969; Spang and Platt, 1972). The data resulting from the studies on enzyme synthesis in the isolated aleurone layer of corn, as a model object in which exogenous gibberellin stands for the phytohormone synthesized in the embryo are in favour of the capability of gene activation. The incubation with GA₃ induces the synthesis of α-amylase as well as of other enzymes (Paleg, 1960; 1965; Filner and Varner, 1967; Jacobsen and Varner, 1967). Jacobsen and Varner (1967) showed that α-amylase synthesis is dependent on RNA formed de novo.

The increment in RNA synthesis may be due to larger number of active genes or to the activation of RNA polymerase. The enhancement in DNA template activity and in the antecedent to it process of stimulation of RNA polymerase activity was found by Jarvis and coworkers in germinating seeds of Corylus avellana (1968). The stimulation of RNA synthesis may also occur as the result of an increase in the activity of the very enzyme and not to the activation of any new genes (McComb et al., 1970; McComb and Brighton, 1972).

The research of Johri and Varner (1968) has shown that in the GA₃-mediated induction of DNA genetic activity might act the substances occurring beyond the nucleus, since the increase in RNA synthesis in the isolated nuclei was found only when the whole procedure had been performed in the presence of the plant hormone. Similarly, the effect of stimulation of ³H uridine uptake was much greater when GA₃ was present during the whole course of plant protoplast isolation (Kleczkowski et al., 1975).

In the light of previous studies on the effects of GA₃ on the process of transcription it is possible to claim that GA₃ acts directly or indirectly on the genetic activity of the nucleus; it seems, however, that there is not only the one way of GA₃ influence on cellular metabolism, and it may paralellly regulate some other processes, as well.

GA₃ strongly stimulates the incorporation of ³H phenylalanine into proteins during the whole interphase in all generations of the antheridial filaments of Chara, the induction of uptake of radioactive amino acid into the cytoplasmic proteins being a little greater than into the nuclear ones. GA₃ stimulates the incorporation of radioactive phenylalanine approximatively in proportion to the intensity of the process in the control; almost the same effect has been reached by the incubation with labeled adenine. These results suggest a low specificity of influence of this plant growth substance on studied processes, since it was impossible to find any connection between the experimental effect and a particular period
of interphase or a particular stage of development in the course of cellular differentiation in the antheridal filaments of Chara.

The stimulation of protein synthesis in the presence of GA₃ was obtained in many objects (ref. G a m b u r g, 1970). It was shown that the enhancement of intensity in the course of the above mentioned process may be due to an increase in both the number and length of polyribosomes (E v i n s, 1971; D a v i e s a n d L a r k i n s, 1973). C h e n and his coworkers (1970) have estimated that protein biosynthesis during early germination in wheat embryos may be prior to RNA synthesis, suggesting an indirect effect of GA₃ on translation and not on transcription.

As results from the estimates of the cell cycle durations and length measurements in the antheridal filaments of Chara, the stimulation of nucleic acids and proteins syntheses does not cause any deviation in the regularity of metabolic processes in these cells. The increase in synthesis intensity has found its positive expression in the enhanced frequency of mitoses in cells having the dimensions a little greater than the control ones. The durations of both the S period and rest part of the cycle: G₂ and mitosis were reduced. The method used did not allow the determination of M duration. Considering that GA₃ accelerated the rate of mitosis in endosperm cells of Hemanthus catherinae in vitro, one can suppose, however, that this hormone stimulates the course of all periods of the cell cycle, i.e. S, G₂, and mitosis (T o m a s z e w s k i, 1973).

The studies on GA₃ effects on processes connected with cell divisions and those determining DNA synthesis were performed on different plants and gave no simple answer as to what is the role of the plant hormone in those processes. GA₃ accelerates root growth in beans owing to the increase in both cell dimensions and their number by the reduction of cell cycle durations (M u r i n, 1974). Similar is the effect of GA₃ on cells of roots and stems of other plants (S a c h s et al., 1957; 1959a; 1961; 1963; P e c k e t, 1960; G u t t i g e a n d T o m p s o n, 1963), as well as on cambial divisions (B r a d l e y a n d C r a n e, 1957).

Although the gibberellin can accelerate the course of the cell cycle, its presence in the medium is not necessary for cell growth and proliferation of tissues cultured in vitro (ref. G a m b u r g, 1970). One can suppose then, that GA₃ is indispensable for the regular metabolism of the cells. It was also impossible to establish the point of special sensibility to the action of the plant hormone in the course of interphase. The research of S a c h s a n d L a n g (1961) indicates that such a point can exist in G₁ period, while that of K i n e t a and his coworkers (1967, cit. after G a m b u r g, 1970) that in G₂. These suggestions do not result from the inhibition of the course of the cell cycle owing to the lack of gibberelin in the medium but they are due to the fact of appearance of mitoses induced by the addition of gibberelin into the culture medium. S a c h s a n d L a n g (1961) have also shown that the hormone increases the number of divisions in subapical stem me-
ristem, but does not induce them. The activation of rosette plants meristems constitutes an exception (Sachs et al., 1959b).

Stimulation of divisions is necessarily associated with the increase in the rate of DNA synthesis. The enhancement of intensity of this process was found in different objects (Madison and Rapaport, 1963; Degani et al., 1970; Durand, 1972; ref. Korableva and Metlin sky, 1973). It was also shown that 5-fluorodeoxyuridine abolishes the induction of elongation due to gibberellin by the process of inhibition of DNA synthesis in stems of pea and seeds of salad (Nitsan and Lang, 1966; Degani et al., 1970). It seems, however, that the increase in rate of DNA synthesis is the by-effect of the plant growth regulator. The study on timings of DNA, RNA, and protein syntheses during pea stem elongation has shown that the stimulation of RNA and protein synthesis is prior to that of DNA synthesis (Giles and Myers, 1966; Broughton, 1969; Gamburg, 1970).

The results presented here and those discussed above of other authors do not allow to demonstrate with what cellular process is connected the effect of GA₃-induced stimulation of the course of the cell cycle and what is its role in the metabolic reactions of the cell. If it is true that this substance is not indispensible for the regular course of the cell cycle, and having regard to the fact of multiplicity of the influence of phytohormone, it appears that the effect of GA₃ consists in general acceleration of all processes necessary for the course of the cell cycle. The results of the present work confirm such an understanding of the action exerted by the growth hormone, since the stimulation of all developmental stages of the antheridial filaments of Chara always occurred in proportion to particular intensities in the control material. Exclusively the cells of 32-celled filaments and to a lesser degree those of 16-celled, that is most advanced in the process of differentiation, appeared to be less sensitive to the influence of this plant growth regulator.

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Udział substancji wzrostowych w regulacji cyklu komórkowego komórek nici spermatogenicznych Chara vulgaris L.

I. Wpływ kwasu gibberelowego na przebieg niektórych procesów w cyklu komórkowym

S tre sc z e n i e

Badano wpływ kwasu gibberelowego (10^{-4} M) na włączanie adeniny 8-{^{14}}C, fenylalaniny ^{3}H w przebiegu intertery, rozmiary komórek mitotycznych i czas trwania faz cyklu komórkowego synchronicznie działających się komórek nici spermatogenicznych Chara vulgaris L. w kolejnych stadiach wzrostu i różnicowania.

GA_{3} stymuluje włączanie obu radioaktywnych prekursorów w całej inter梅西e i we wszystkich stadiach rozwojowych nici w przybliżeniu proporcjonalnie do poziomu włączania w kontroli. Gibberolina ta nieznacznie zwiększa rozmiary komórek i silnie skracza czas trwania cyklów komórkowych — w podobnym stopniu fazę S i G_{2} + M. Działanie to jest większe we wcześniejszych stadiach rozwojowych nici spermatogenicznych.

Przeprowadzone badania nie pozwoliły na ujawnienie okresu intertery szczególnie wraźliwego na GA_{3}, gibberolina ta stymulowała bowiem przebieg wszystkich badanych procesów, co skłania do interpretacji jej działania jako niespecyficzego stymulatora metabolizmu komórek nici spermatogenicznych u Chara vulgaris L.