Studies on the activity of kinetin in cultures of *Funaria hygrometrica*

II. Effect of kinetin on isolated protonema cells

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In cultures of the protonema of mosses, kinetin may induce bud formation without stimulating the growth of the culture (Szweykowska and Handszu 1965; Szweykowska, Ratajczak and Schneider 1968). This suggests a more or less direct role of this factor in the bud-forming process. The locus of bud induction is a single protonemal cell which, however, belongs to a protonema of a determined size and age. To obtain information on the effect of kinetin on isolated protonema cells, single cells were excised from protonemas grown in nutrient solutions with or without kinetin and their development in similar media was observed.

MATERIAL AND METHODS

Spore capsules of *Funaria hygrometrica* were sterilized with 0,2% HgCl_2 , and spores inoculated on liquid media were grown under continuous light at 22—25°C. As basal medium, Kofler's inorganic solution was used (Szweykowska and Handszu 1965). Organic media were supplemented with 0,25 per cent glucose. Kinetin was added to the media in a dose of 0,1 and 1 mg/l. After 10 days, single apical cells were excised from the protonema filaments and transferred to depression slides with drops of medium of the same composition. The slides were covered with cover slips tightened with vaseline and kept in similar conditions of light and temperature as the protonema cultures. The cells were observed in microscope every day for a 15-days period. Ten drop cultures were used for each experimental variant.

Some cells were fixed in aceto-ethanol for 48 hrs., passed through the ethanol series to water, placed in 4 per cent iron alum for 15 min., washed with water and stained for 5 min. with propiono-carmin.

RESULTS

Ten days after inoculation of spores the cultures consisted of protonema with no buds as yet, although the organic cultures with kinetin were almost ready for bud formation. The cells were filled with chloroplasts assembled particularly along the cell walls.

In the inorganic control medium, the excised apical cells were 60—130 μ long and 10—14 μ wide. In the presence of kinetin, the cells were wider (15—20 μ). The cells excised from protonema grown in organic control medium were 100—135 μ long, i.e. about as long as in the inorganic control, but wider (17—22 μ). In the presence of kinetin, the cells from organic cultures were considerably longer (180—200 μ in 1 mg/l solution).

In the inorganic control medium, the first division of the cell occurred around the 3rd day, and growth and divisions ceased after 9 days of drop culture. In the resulting filament, the apical cell always divided, and no divisions of intercalary cells were observed (figs. 4—6). Branching was observed only in two out of ten filaments. The nuclei which could be observed in cells stained with propiono-carmin were very small (fig. 1).

Kinetin (1 mg/l) considerably stimulated cell divisions which began on the first day and continued till the end of the experiment. It caused also divisions of intercalary cells and abundant filament branching with a tendency to inhibition of the filament growth in length (fig. 7). The 3—5 cells long filament did not increase any more in length. After about 7 days, characteristic protrusions appeared in both apical and intercalary cells which after formation of a wall separating them from the main filament developed into perpendicular side branches. The cells of these branches divided every day and caused their elongation. Frequently, the cells doubled their width and became spherical. Kinetin considerably increased the size of the nuclei which in 1 mg/l solution were about twice as large as in the control (figs. 1—3).

Figs. 1—3: Cells from inorganic medium, fixed and stained with propiono-carmin: 1 — control, 2 — kinetin 0.1 mg/l, 3 — kinetin 1 mg/l. 4—6: Inorganic control medium, 4 — excised cell, 5 — after 3 days, 6 — after 15 days. 7: Inorganic medium with 1 mg/l. kinetin, after 15 days. 8—9: Organic control medium, 8 — excised cell, 9 — after 15 days. 10 — Organic medium with 1 mg/l. kinetin, after 15 days.

Fig. 1—3: Komórki z pożywki nieorganicznej utrwalone i barwione karminem propionowym: 1 — kontrola, 2 — kinetyna 0.1 mg/l, 3 — kinetyna 1 mg/l. 4—6: Nieorganiczna pożywka kontrolna, 4 — komórka wyjściowa, 5 — po 3 dniach, 6 — po 15 dniach. 7: Pożywka nieorganiczna z dodatkiem kinetyny 1 mg/l, po 15 dniach. 8—9: Organiczna pożywka kontrolna, 8 — komórka wyjściowa, 9 — po 15 dniach. 10 — Pożywka organiczna z dodatkiem kinetyny 1 mg/l, po 15 dniach.

Fig. 1—10

In the organic control medium, the chloroplasts and the nuclei were larger, and the divisions more frequent than in the inorganic control. Besides apical, also intercalary cells divided. No side branches were observed (figs. 8—9).

In organic medium containing kinetin (1 mg/l), numerous divisions of both apical and intercalary cells occurred every day resulting in a quick elongation of the filament (fig. 10). After 10 days, side protrusions appeared in older cells which soon developed into branches. Most cells were elongated, but short cells were also observed. After 10 days, the cells ceased to grow and divide. Kinetin also slightly increased the size of the nuclei which were about as large as in inorganic medium with 1 mg/l. kinetin (fig. 3).

In spite of 15 days long cell culture in drops of nutrient solutions containing kinetin, no bud formation was observed in any case, although in organic cultures inoculated with spores buds appear after about 10 days.

DISCUSSION

Bopp and Diekmann (1967) isolated short filaments from the caulonema of *Funaria hygrometrica* cultures grown on agar and obtained bud formation in filaments only 5 cells long after transferring them on a kinetin medium. However, the bud formation depended on the age of the caulonema and also decreased considerably when isolated filaments were placed for some time on kinetin-free medium before being exposed to kinetin action. This led Bopp and Diekmann to the conclusion that for bud formation a second factor cooperating with kinetin is necessary.

In the present experiments, we used single apical chloronema cells, as typical caulonema forms do not occur in liquid cultures which, nevertheless, are able to form buds. In these experiments, kinetin acted as an inducer of cell divisions and filament branching, cell growth and nucleus enlargement. No bud formation was obtained even from cells originating from organic kinetin cultures and grown further in the same (however fresh) medium, i.e. in conditions and at a time meeting the requirements for bud formation. This supports the conclusion of a requirement for a second, probably more specific bud-inducing agent. Some evidence for the existence of such an agent was found in the investigations of Bopp and Klein (1963) and of Klein (1967) on "factor H" produced by the protonema and responsible for bud formation. This process begins with localized cell divisions in the protonema, with a change of their orientation which results in a three-dimensional growth, and with the origin of the organizing pyramidal apical cell.

Cytokinins seem primarily to be involved in the preparatory cell division step of this process, and their occurrence in mosses has recently been demonstrated by Bauer (1966). The further steps of bud formation probably require additional factors, and stimulation of their synthesis may be a secondary effect of kinetin, in view of the promoting action of kinetin on RNA synthesis in protonema cells (Brandes 1967) and the presence of cytokinins in t-RNA preparations (Skoog et al. 1966) which indicate an essential role of cytokinins in protein and enzyme synthesizing systems.

SUMMARY

Single apical cells were excised from the protonema of control and kinetin cultures of the moss *Funaria hygrometrica*, transferred to drops of fresh media and observed for 15 days. Kinetin in 1 mg/l. concentration considerably stimulated cell divisions which occurred in both apical and intercalary cells and caused abundant filament branching. In no case were gametophore buds obtained even from cells originating from organic kinetin cultures which at the time of cell excision were mature to bud formation. It is concluded that cytokinins determine a preliminary cell division step of bud induction, and that for further steps additional factors are probably required.

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Badania nad aktywnością kinetyny w kulturach Funaria hygrometrica

II. Wpływ kinetyny na izolowane komórki splećka

Streszczenie

Ze splećka mchu *Funaria hygrometrica* rosnącego na pożywkach z kinetyną i bez kinetyny izolowano pojedyncze szczytowe komórki, przenoszono je do kropli świeżych pożywek i obserwowano przez 15 dni. Kinetyna w stężeniu 1 mg/l stymulowała silnie podziały komórkowe wywołując je zarówno w komórkach szczytowych, jak i środkowych, ponadto powodowała znaczne rozgałęzianie się nici splećkowej. W żadnym przypadku nie uzyskano wytworzenia pączków gametoforowych, nawet z komórek pochodzących z kultur na pożywce organicznej z kinetyną, które w momencie izolowania komórek były dojrzałe do tworzenia pączków. Z doświadczeń wysunięto wniosek, że cytokininy kontrolują wstępne stadium indukcji pączków polegające na zlokalizowanym wzmożeniu podziałów komórkowych w splećku. Dla dalszych stadiów procesu pączkotwórczego prawdopodobnie niezbędne są dodatkowe czynniki.