

## Cytokinin-induced decrease in ribonuclease activity and initiation of gametophore buds in the protonema of mosses

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### Abstract

As early as after 4 hours of kinetin treatment a decrease in RNase activity was found in the moss protonema and it was maintained to at least 10 hours. It was shown that this decrease was correlated with the morphogenetic effect of kinetin (bud induction). No allosteric inhibition of RNase by kinetin could be found. The decrease in enzyme activity was more pronounced when additionally inhibitors of protein and RNA synthesis were used. It is concluded that kinetin affects the RNase rather by an inhibition of *de novo* synthesis of the enzyme than by an increase of its decomposition by proteases.

### INTRODUCTION

Initially a decrease and later a strong increase in ribonuclease activity occur during cytokinin-induced process of gametophore bud formation in a moss protonema (Schneider and Szweykowska, 1974). In our previous paper (Spychała et al. 1975) it has been reported that similar changes in enzyme activity occur during gametophore formation in normal ontogenesis, not affected by exogenous regulators. It has also been found that the increased activity of RNase in the second phase of cytokinin action is distinctly related to its morphogenetic effect.

As the metabolic changes preceding the morphogenetic transformations are of particular importance, in this paper results are presented of a more detailed investigation of the early decrease in RNase activity in cytokinin-treated protonemata.

## MATERIAL AND METHODS

Sterile protonema cultures of two moss species were used in experiments, *Ceratodon purpureus* (Hedw.) Brid. and *Funaria hygrometrica* (L.) Sibth. The protonemata of *Funaria* were grown from spores and those of *Ceratodon* from vegetatively propagated protonemal stock culture. Composition of the medium and culture conditions were described in our previous paper (Spychała et al. 1975). Ribonuclease activity was determined by a modified method of Tuve and Anfinsen (Schneider and Szweykowska, 1974).

## RESULTS

## Changes in RNase activity during incubation of the protonema with kinetin

Schneider and Szweykowska (1974) described changes in RNase activity after incubating the protonema of *Ceratodon purpureus* with kinetin for 24, 48 and 120 hours. For a more detailed investigation of the time-course of these changes, the activity of enzyme was determined at shorter time intervals, with a special attention to short incubation times. Thirty-day-old protonema cultures of *Ceratodon* were transferred from stock agar medium to a liquid medium of the same composition (with the omittance of agar) to which kinetin at a bud-inducing concentration of 5  $\mu$ M was added (no addition in control medium). During the first 12 hours of experiment, samples of the protonema were taken every 2 hours, and next after 24, 48 and 120 hours. RNase estimations were accompanied by morphological observations of the protonema. After 24 hours of treatment, kinetin induced formation of bud initials, and after 48 hours a large number of several-celled gametophore buds was observed. The strongest bud-inducing effect of kinetin could be observed after 120 hours. As early as after 4 hours of kinetin treatment a decrease in RNase activity was found. The lowered activity of enzyme was maintained at least up to 10 hours, and afterwards the activity gradually increased, to a level more than twice as high as that of the control (Fig. 1a). Similar results were obtained in experiments with 10-day-old liquid cultures of *Funaria hygrometrica*, in which the early decrease in RNase activity was even stronger, and after 120 hours of kinetin treatment, when great numbers of buds were present in cultures, the activity of enzyme amounted also to 200% of control (Fig. 1b).

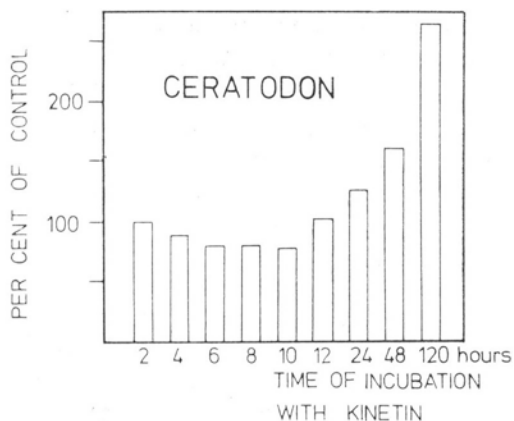


Fig. 1a. Time-course of changes in RNase activity during incubation of the protone-ma of *Ceratodon purpureus* with kinetin in conc. of  $5 \mu\text{M}$ . Thirty-day-old protone-mata were transferred from stock agar medium to a liquid medium with and without kinetin resp. RNase activity was determined by a modified Tuve and Anfinsen method.

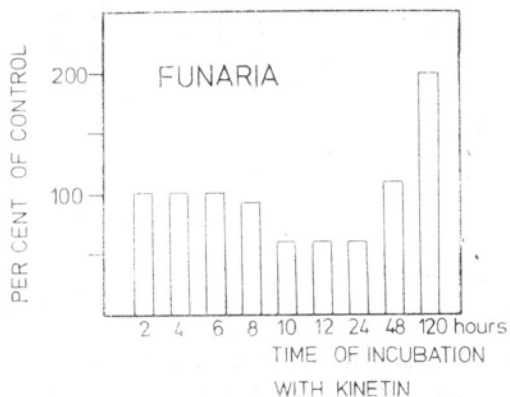


Fig. 1b. Time-course of changes in RNase activity during incubation of the protone-ma of *Funaria hygrometrica* with kinetin in conc. of  $0.5 \mu\text{M}$ . Kinetin was added to 10-day old protonema cultures grown from spores. RNase was determined by a modified Tuve and Anfinsen method.

#### Relationship between decrease in RNase activit yand bud formation

Decrease in RNase activity after 4–10 hours of incubation of the protonema with kinetin can be a direct molecular effect of this regulator on the protonema of mosses. To test whether there is a correlation between this effect and the induction of gametophore buds, experiments were carried out in which advantage had been taken of the fact that *Ceratodon* forms buds under the influence of kinetin in light as well as in the dark, whereas *Funaria* shows no bud-formation response to kinetin in darkness.

RNase activity was examined in protonemata of the two species in light and dark conditions after 10 hours of kinetin treatment.

In light conditions, a decrease in RNase activity was found in both *Ceratodon* and *Funaria*. In protonemata left in kinetin medium for next 110 hours a strong bud-inducing effect was observed. In the dark, the decrease in RNase activity was found in the protonema of *Ceratodon*, but not in that of *Funaria* (Fig. 2). Correspondingly, only in the protonema of

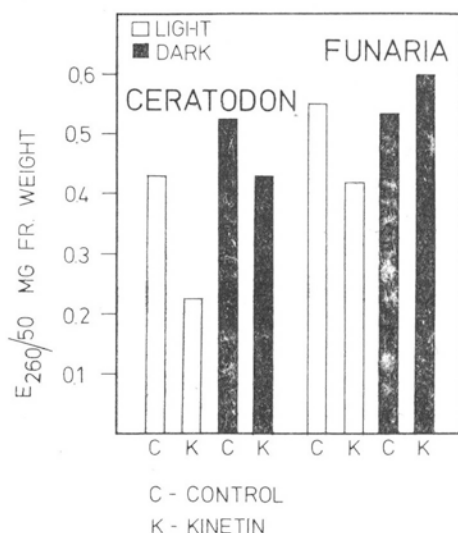


Fig. 2. Effect of 10 hours of kinetin treatment on the RNase activity in light and dark conditions. Prior to the kinetin treatment, the protonemata were cultured from spores on media containing glucose at 0.25% (*Funaria* in liquid medium for 10 days, and *Ceratodon purpureus* on agar medium for 14 days and than in liquid medium for 7 days, in light conditions. Activity of the enzyme was determined by a modified method of Tuve and Anfinsen

C — control; K — kinetin: 6.5  $\mu$ M for *Funaria hygrometrica*, 5  $\mu$ M for *Ceratodon purpureus*

*Ceratodon purpureus*, when it was left for next 110 hours in kinetin medium and under dark conditions, the bud-inducing effect appeared, whereas it was absent in *Funaria* under the same conditions. The experiments thus showed that the decrease in RNase activity occurring in the protonema in the early phase of kinetin action is correlated with its morphogenetic effect.

#### The allosteric effect of kinetin and the effect of inhibitors of protein and RNA synthesis on RNase activity

The decrease in RNase activity in the presence of kinetin might result from a reduced synthesis of the enzyme, from its accelerated breakdown

by way of raising the level of proteases or from an allosteric inhibition. To obtain some information on this question, two series of experiments were carried out. In the first one, kinetin at 5  $\mu\text{M}$  (the bud-inducing concentration) was added to a crude extract from the protonema of *Ceratodon*, and immediately afterwards RNase activity was estimated. No change in enzyme activity was found when compared with the sample without kinetin. Lower kinetin concentrations (0.5, 0.05, 0.005, 0.0005  $\mu\text{M}$ ) were also without effect. Thus the possibility of kinetin acting as an allosteric inhibitor of the enzyme has not been confirmed. (Table 1).

Table 1

The allosteric effect of kinetin. Kinetin was added to a crude extract from the protonema of *Ceratodon purpureus* and immediately afterwards the RNase activity was estimated

RNase activity $E_{260}/50 \text{ mg fr. weight}$		
1	Control	0.270
	Kinetin 5 $\mu\text{M}$	0.270
2	Control	0.420
	Kinetin 5 $\mu\text{M}$	0.420
3	Control	0.300
	Kinetin 5 $\mu\text{M}$	0.280
4	Control	0.300
	Kinetin 5 $\mu\text{M}$	0.300
	Kinetin 0.5 $\mu\text{M}$	0.290
	Kinetin 0.05 $\mu\text{M}$	0.290
	Kinetin 0.005 $\mu\text{M}$	0.310
	Kinetin 0.0005 $\mu\text{M}$	0.320

In the second series of experiments, following inhibitors of protein and RNA synthesis were used: m-fluorophenylalanine (100  $\mu\text{M}$ ), acetylleucine (500  $\mu\text{M}$ ) and azaadenine (500  $\mu\text{M}$ ). In these concentrations, the inhibitors completely but reversibly inhibited the bud-inducing effect of kinetin. Inhibitors were added with and without kinetin. After 10 hours of treatment, they produced a decrease in RNase activity. It was more pronounced when additionally kinetin was present in the medium, in fact, the inhibitory effect of antymetabolites and kinetin were summed up (Fig. 3). One can conclude that kinetin affects the RNase rather by an inhibition of de novo synthesis of the enzyme than by an increase of its decomposition by proteases.

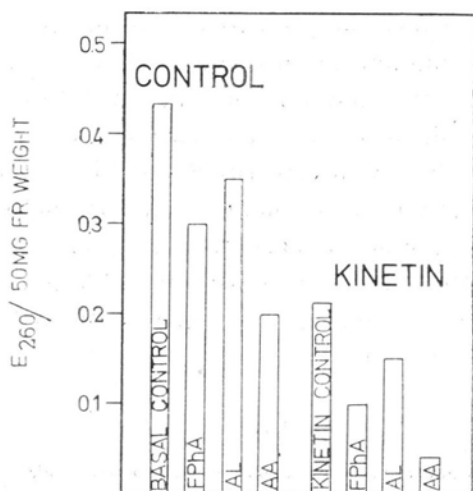


Fig. 3. Effect of inhibitors of protein and RNA syntheses on the RNase activity in the protonema of *Ceratodon purpureus* treated or non-treated with kinetin 5  $\mu\text{M}$ . Ten-day-old protonemata were transferred from agar to liquid media with and without kinetin and with the addition of antimetabolites:

PFha — fluoro phenylalanine of 100  $\mu\text{M}$ ; AL — acetylsoleucine at 500  $\mu\text{M}$ ; AA — azaadenine at 500  $\mu\text{M}$ .

## DISCUSSION

While the morphological development of kinetin-induced gametophore buds is most pronounced after five days of incubation of the protonema with the regulator, changes in the metabolism are detectable after much shorter time. Using a cytochemical method, Brandes (1967) found an accumulation of RNA in the "pro-bud" cells after 10 hours of kinetin treatment. Schneider and Szweykowska (1975) showed increase in the activity of t-RNA-nucleotidyltransferase in the protonema after 1–3 hours of treatment with 6- $\Delta^2$ -isopentenylaminopurine. In this study, the first changes in RNase activity were found in the protonema as early as after 4 hours of kinetin treatment. Similarly as in the case of the increase in RNase activity after a longer time of kinetin treatment (Spychała et al. 1975), also the decrease in the activity of the enzyme was distinctly correlated with the later bud-induction. This may be a significant feature for the biochemical characterization of the early stages of the formation of gametophore buds. A lowering by cytokinins of RNase activity seems in particular to be a characteristic property of this group of growth substances (Srivastava and Ware 1965, Sodek and Wright 1969, Dove 1971).

An important question is the mechanism of RNase inhibition by cytokinins. The negative results from experiments on the allosteric effect of kinetin suggested that the regulator does not act as an allosteric

effector, although such an effect in increasing the RNase and DNase activity was shown in bean hypocotyls by Maciejewska-Potapczyk (1959). It is also little probable that the kinetin-induced decrease in RNase level was a result of an increased level of proteolytic enzymes in protonema cells, as a decrease in RNase caused by inhibitors of RNA and protein syntheses was still intensified by kinetin. A direct inhibition of RNA synthesis by kinetin seems thus to be the most probable explanation.

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*Obniżenie poziomu rybonukleazy przez cytokininę a inicjacja pąków gametoforowych w spletku mchów*

#### Streszczenie

Już po 4 godzinach działania kinetyny stwierdzono w spletku mchu obniżenie aktywności RNazy, które utrzymywało się do 10 godzin. Wykazano korelację między

tym obniżeniem a morfogenetycznym efektem kinetyny (indukcja pąków). Użycie inhibitorów syntezy białka i RNA pogłębiło hamujący wpływ kinetyny na RNazę, przy czym nie wykazano allosterycznego działania tej substancji. Przypuszcza się, że kinetyna obniża poziom RNazy raczej na drodze hamowania syntezy cząsteczek enzymu *de novo*, aniżeli przez zwiększenie ich rozkładu za pomocą proteaz.