Morphological return from mitotic prophase to interphase induced by RNase in plant cells

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Summary. In vivo treatment of prophasic meristematic cells with RNase shows that the cytological reaction is similar to that produced by protein synthesis inhibitors, and that the prophasic control of protein synthesis is earlier than the region where prophasic RNA is synthesized.

To elucidate the part played by RNA and proteins in the various stages of the cell cycle, numerous investigations have been carried out to study the effects produced by an inhibition of RNA and protein synthesis, using a variety of materials and inhibitors[1,2].

The ability of ribonuclease (RNase) to produce mitotic abnormalities has been reported by several authors[3,4]. The purpose of the present paper is to study the effect of RNase as an inhibitor of protein synthesis on plant prophase.

Material and methods. The material used was the root meristems of Allium cepa L. The condition of the cultures, the cytological labelling, and stained methods were described in a previous paper[5]. – To evaluate the efficiency of the RNase, different bulbs were incubated with 0.02 μCi/ml 10^{-4} M ^{14}C-leucine (with a specific activity of 344 μCi/mM). They were considered control bulbs, while others were incubated after labelling in the same way in solutions containing RNase from bovine pancreas, 5 × crystallized, type 1-A (Sigma) at different concentrations (100, 500 and 1000 μg/ml). The concentrations (mg) of the ^{14}C-leucine labelled protein (cpm) were determined in each case as previously described[5].

Results and discussion. 1. Comparative effects of RNase and an RNA synthesis inhibitor on mitotic prophase. González-Fernández et al.[6,7] used RNA synthesis inhibitors to demonstrate the existence of RNA synthesized during prophase which is involved in plant mitosis progression. In order to compare the effect of RNase and that of an RNA synthesis inhibitor such as 3'AdR (cordycepin) on mitotic prophase synchronous populations, we used synchronous populations induced by a caffeine treatment 0.1% during 1 h.

When the binucleate population initiated biprophase, the roots were subjected to continuous treatment with RNase or cordycepin (figure 1). This figure shows that cordycepin produces an accumulation and blockage of cells, the percentage of biprophases remains constant with time, while in the presence of RNase no prophasic accumulation is observed, the representative curve peaking and subse-

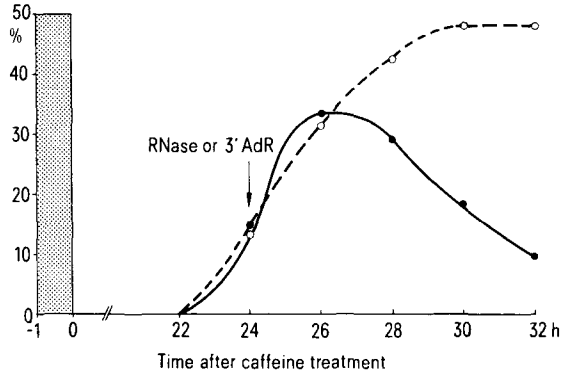

Fig. 1. Evolution of the percentage of biprophases observed 24 h after the end of caffeine treatment on initiation of continuous treatment with RNase 500 μm/ml of cordycepin 10^{-4} M. Ordinatae: % of binucleate cells in prophases. Abscissae: hours after caffeine treatment. Shaded area: 1 h 0.1% caffeine treatment. ● Biprophases in the presence of RNase; ○ biprophases in the presence of cordycepin.

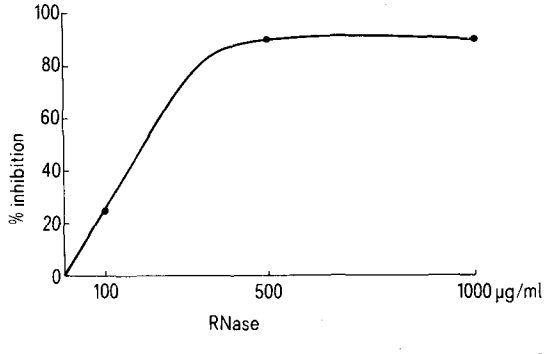

Fig. 2. Effect of various concentration of RNase on protein synthesis.

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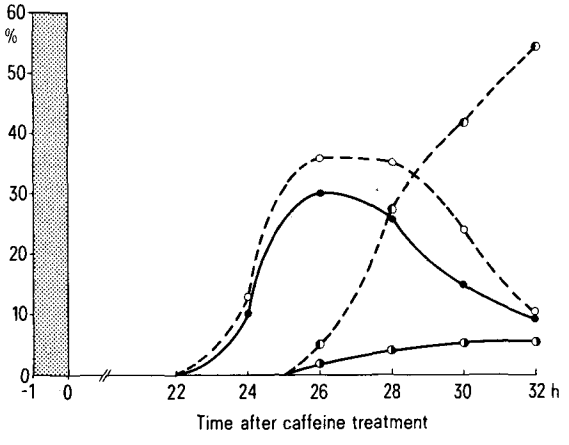

Fig. 3. Effect of treatment with colchicine 0.1% and RNase 500 μg/ml plus 0.1% colchicine as from the 24th h after the end of caffeine treatment. Ordinate % of binucleate cells. Abscissae: time in hours after termination of caffeine treatment. Shaded area: 1 h 0.1% caffeine treatment. ●% of biprophases in the presence of RNase + colchicine; ○ % of biprophases in the presence of colchicine; ◑ % of bimetaphases in the presence of RNase + colchicine; ◐ % of bimetaphases in the presence of colchicine.

quently falling off. This type of curve is similar to that observed when treatment is carried out with protein synthesis inhibitors under the same experimental conditions[5].

2. Evaluation of the inhibitory capacity on protein synthesis. In order to test the effect of RNase on protein synthesis, 3 concentrations were tested: 100, 500 and 1000 µg/ml. The criterion used to quantify protein synthesis inhibition was to consider the radioactivity observed in the control samples as 0% inhibition. The results obtained are set out in figure 2 from which we may deduce that 500 µg/ml of RNase is a suitable concentration, as it gives 90% inhibition of total protein synthesis.

3. Cytological study of RNase as a protein synthesis inhibitor in mitotic prophase. The inhibitory effect of RNase on protein synthesis in mitotic prophase was observed by using a synchronous binucleate population produced by caffeine and RNase 500 µg/ml from the 24th h after the end of caffeine treatment. Together with the enzyme, we administered colchicine 0.1% in continuous treatment in order to observe the metaphase accumulation.

Figure 3 shows the behaviour of the binucleate population on the presence of RNase-colchicine, and a comparison is made with its behaviour in the presence of colchicine alone. The same figure demonstrated how a similar curve is obtained from the evolution of the relative number of biprophases, both in control samples with colchicine and in the samples treated with enzyme-colchicine. The bimetaphase curve, however, is markedly different. These results strongly suggest that prophase in the presence of RNase neither go through to metaphase nor remain blocked in prophase but rather regress to interphase.

Using experiments combining protein synthesis inhibitors and RNA synthesis inhibitors, region of return, was situated earlier than the region where prophasic RNA is synthesized (blocking region). The experiments described in this paper themselves reafirm the earlier demonstration, since RNase acts simultaneous as a RNA digestive enzyme and as a protein synthesis 'inhibitor'. The response of the synchronous population to RNase treatment is the same as its response to protein synthesis inhibitors.

On the other hand, we observed no inhibition of metaphase, anaphase and telophase processes nor of cytokinesis, which would suggest that these events are dependent neither on RNA nor, in the short term, on protein synthesis.

Free choline in sea urchin embryos (Paracentrotus lividus)

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Summary. A sensitive radio-enzymatic assay for choline, applied to extracts of developing sea urchin embryos, revealed that free choline in eggs declines during cleavage and rises abruptly previous to gastrulation, indicating an important shift in choline metabolism between these two stages.

Choline has three major roles in cellular physiology and biochemistry. First choline occurs in animal cell membranes as phosphatidyl choline, a principal component of both plasma and mitochondrial membranes. A second function of choline depends on its oxidation by choline oxidase to betaine, an important source of methyl groups in biological methylations. A third role for choline is as precursor for acetylcholine formed in the presence of choline acetylase.

While mammalian cells receive their required choline from their medium, embryos developing independently of exogenous nutrients contain phospholipid reserves in yolk and other organelles and these reserves are metabolized progressively during early development. In sea urchin embryos although the net amount of phospholipids is constant during early development the synthesis of phospholipids increases suggesting that new phospholipids are made from the degradation products of those in reserve[1].

Since knowledge of the quantities of free choline available to embryos during development could tell us something about their regulation of choline metabolism we have measured the freely extractable choline from whole embryos by means of a highly sensitive and specific assay and found important changes in the amounts of free choline available from embryos at different stages.

Materials and methods. Eggs and embryos of the sea urchin *Paracentrotus lividus* were extracted in lots of 5000 embryos at various developmental stages in perchloric acid and neutralized. Dried extracts were assayed by a radioenzymatic assay dependent on choline kinase[2]. Choline kinase was prepared as described by ACARA and RENNICK[3].

Fifty µl of the incubation medium consisting of 10 m*M* *Tris* buffer at pH 9.5, 5 m*M* $MgSO_4$, 0.1 m*M* ATP, 2 µg of purified choline kinase and 1 µCi of ^{32}P-ATP were mixed with 1 to 10 µl of dissolved extract from the embryos. The samples were incubated 3 h, chilled and 10 µl of 0.045 *M* phosphoryl choline were added to each tube and the entire mixture placed on 2 ml columns of BioRad AG 1-X8 (200–400 mesh) which had been equilibrated with 10 m*M* *Tris*, pH 11 in 10 m*M* $MgSO_4$, 10 m*M* NaF and 30 m*M* NaCl. The labelled phosphoryl choline formed by the reaction of choline with ^{32}P-ATP in the presence of choline kinase was eluted from the column with 7 ml of *Tris* buffer at pH 11, collected in scintillation vials, and the radioactivity determined by Cerenkov counting. Triplicate samples of each sea urchin extract, with and without added choline, were analyzed to test for any inhibition of the assay system caused by the extract.

Results and discussion. The developmental pattern of the free choline in sea urchin embryos is shown in the Figure. The unfertilized eggs contain 0.2 picomoles choline per egg, which constitutes a relativly large reser-

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