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REGULATION OF POLYRIBOSOME FORMATION AND CELL DIVISION IN CULTURED SOYBEAN CELLS BY CYTOKININ

K. C. SHORT,* D. A. TEPFER AND D. E. FOSKET†
Department of Developmental and Cell Biology, University of California, Irvine, California 92664, *U.S.A.*

SUMMARY

Cytokinin was shown to be required for cell division in cultured cells of *Glycine max* var. Sodifury. This cytokinin-induced mitotic activity was correlated with a high cellular content of polysomes. Within 24 h after transfer to a cytokinin-containing medium there was a 4·5-fold increase in the percentage of ribosomes bound as polyribosomes, as determined by sucrose density gradient centrifugation of extracted ribosomal material. Relatively high levels of polysomes and mitotic activity were maintained through the first 6 days of the culture period on cytokinin-containing medium. Thereafter, both cell division activity and the percentage of polyribosomes declined progressively with increasing time in culture. A comparatively small increase in polyribosomes occurred within 24 h of transfer to medium lacking cytokinins, followed by the progressive decline of the level of polyribosomes. The time course of cytokinin-induced polyribosome formation was determined by treating cells with cytokinin after they had been cultured for 24 h on a medium lacking cytokinin. Under these conditions there was a rapid increase in polyribosomes over the next 3 h with no detectable lag period, and near maximal levels of polyribosomes after 6 h of treatment. The initial stimulation of polyribosome formation by cytokinin was not blocked by actinomycin D. Cytokinin was shown to have a comparatively small effect on the ribonuclease activity in extracts of these cells.

INTRODUCTION

Cytokinins are plant cell division factors. They are naturally occurring or synthetic compounds which elicit cell proliferation in certain plant tissues when these tissues are cultured *in vitro* on a medium which also contains an auxin (Miller, Skoog, von Saltza & Strong, 1955; Skoog & Miller, 1957; Torrey, 1961; Letham, 1967). Cytokinins appear to play a rather specific role in regulating events in the mitotic cycle. Cultured soybean and tobacco cells will undergo DNA synthesis in the absence of a cytokinin, but they will not undergo mitosis after replicating their DNA unless cytokinin is present (Das, Patau & Skoog, 1956, 1958; Patau & Das, 1961; Jouanneau & Tandeau de Marsac, 1973; Fosket & Short, 1973). These results suggest that cytokinins regulate the mitotic cycle by permitting critical events to occur either in the G_2 phase of the cell cycle, or in the transition from G_2 to mitosis.

Cytokinins have been shown to stimulate protein synthesis in some plant tissue (Atkin & Srivastava, 1970; Trewavas, 1972). Furthermore, protein synthesis has been

* Present address: North East London Polytechnic, Romford Road, London, England.
† To whom requests for off-prints, etc. should be made.

shown to be necessary for the progress of cells through their division cycle (Jones, Kates & Keller, 1968; Parchman & Stern, 1969; Webster, 1973). Therefore, we have examined the effect of cytokinin on protein synthesis in a strain of cultured soybean cells in which cell division is dependent upon cytokinin in order to determine the relationship between cytokinin-induced mitotic activity and protein synthesis. Preliminary work indicated that it would be difficult to measure the rate of protein synthesis in these cells by determining the rate of labelled amino acid incorporation into protein *in vivo* because cytokinin treatment influenced the rate of amino acid uptake and the pool sizes of the amino acids, as well as the rate of precursor incorporation. Since polyribosomes have been shown to be the machinery of protein synthesis and since the polyribosome content of many cells has been shown to be directly correlated with the rate of protein synthesis in these cells (Wettstein, Staehelin & Noll, 1963; Payne, Brownrigg, Yarwood & Boulter, 1971; Travis & Key, 1971), we have examined the effect of cytokinins on the polyribosome content of cultured soybean cells. Our results demonstrate that cytokinin treatment rapidly stimulated polyribosome formation and that mitotic activity is correlated with a high polyribosome content.

MATERIAL AND METHODS

Soybean tissue culture

Callus tissue, originally derived from the cotyledons of *Glycine max* var. Sodifury, was maintained on a modification of Miller (1961) medium designated SCF (Fosket & Torrey, 1969). The tissue was routinely subcultured at 3-week intervals and grown at 23 °C under continuous, diffuse fluorescent light. For experimental purposes, small, relatively uniform explants (approximately 15 mg fresh weight) were cut from 21-day-old, stationary phase tissues; 10 explants were either placed in a 6-cm diameter plastic Petri dish containing 20 ml of agar solidified medium, or 20 ml of liquid medium. Short-term liquid cultures in Petri plates were placed on a reciprocal shaker completing 20 cycles/min.

Polyribosome isolation

Soybean callus (1·5–3·0 g fresh weight) was frozen on dry ice and ground in a precooled mortar and pestle. The fine powder was suspended in extraction medium (0·25 M sucrose, 50 mM *N*-2-hydroxy-ethylpiperazine-*N*-ethanesulphonic acid [HEPES] buffer, pH 7·8, 0·4 M KCl, 20 mM magnesium acetate, 7 mM β-mercaptoethanol, 1 mM dithiothreitol and 0·1 % diethyl pyrocarbonate [diethyl oxydiformate]). The mixture was stirred gently for 3 min and filtered through Miracloth to remove cell walls and unbroken cells. The filtrate was centrifuged at 13000 *g* for 15 min at 4 °C to remove nuclei, starch grains and wall fragments. A ribosomal pellet was obtained from the supernatant by layering the sample over a discontinuous sucrose gradient consisting of 1·0 ml of 0·5 M and 1·0 ml of 1·5 M sucrose containing 50 mM HEPES (pH 7·8), 0·4 M KCl, 20 mM magnesium acetate, 7 mM β-mercaptoethanol and 1 mM dithiothreitol, followed by centrifugation at 165000 *g* in a Beckman titanium 50 rotor for 3 h at 4 °C. The pelleted ribosomes were gently resuspended in 1 ml of 50 mM HEPES buffer (pH 7·8) containing 0·2 M KCl and 10 mM magnesium acetate. Aliquots of the suspended ribosomes were layered on to continuous, linear sucrose gradients (5–35 %) containing 50 mM HEPES (pH 7·8), 0·2 M KCl, and 10 mM magnesium acetate, supported by a 0·5-ml cushion of 45 % sucrose, and centrifuged at 190000 *g* for 35 min in an SW50 rotor at 4 °C. After centrifugation, the bottom of the tubes was punctured with a hypodermic needle and 45 % sucrose was pumped into the tubes, forcing the gradients out through a tube at the top and into a flow cell where optical density at 260 nm was determined with a Beckman Acta III recording

spectrophotometer. The base line was determined by fractionating a blank gradient. Changes in the amount of polyribosomes relative to monoribosomes were determined by measuring the area under the polyribosomes and monoribosome regions of the absorbancy profiles.

Ribonuclease activity

The assay was a modification of the methods of Lyndon (1966) and Birmingham & Maclachlan (1972). About 0·5 g of tissue was homogenized in 2 ml of polysome extraction buffer, as described above, at 0–4 °C and then centrifuged at 10000 *g* for 10 min. The supernatant was retained and made up to a known volume with extraction buffer. The reaction was initiated by adding 0·2-ml aliquots of extract to 0·6 ml of 0·25 % wheat germ RNA (highly polymerized, Calbiochem) in 0·2 M Tris-HCl buffer, pH 6·0. The mixture was incubated at 37° C for 1 h and the reaction terminated by adding 1·0 ml of 0·38 % (w/v) uranyl acetate dissolved in 10 % trichloroacetic acid containing 10 % of concentrated HCl. The reaction mixture was placed in an ice bath for 30 min, after which it was centrifuged at 20000 *g* for 10 min. RNase activity was indicated by an increase in the $E_{260\ nm}$–$E_{290\ nm}$. An arbitrary standard of RNase activity was used in which an increase in $E_{260\ nm}$–$E_{290\ nm}$ of 0·014 units was taken to be equivalent to the hydrolysis of 1 μg of RNA. RNase activity was expressed as μg RNA hydrolysed/mg of protein in the enzyme extract. The protein content of the extracts was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

The importance of cytokinin for mitotic activity by cultured soybean cells is illustrated by the data presented in Table 1. Five-day-old soybean cells, with a mitotic index of approximately 3·5, were transferred to SCF medium which either contained or lacked the cytokinin zeatin. Without zeatin, the mitotic index dropped to 0·19 within 24 h, and it reached zero by the 3rd day of culture. When the same tissue was transferred to SCF medium containing 5×10^{-7} M zeatin, the mitotic index

Table 1. *Changes in the mitotic index of soybean tissues with time in culture*

Time after inoculation, days	Mitotic index: Tissues cultured on cytokinin-containing medium	Mitotic index: Tissues cultured on medium lacking cytokinin
1	6·91	0·19
2	4·10	0·11
3	3·28	0
4	3·20	0
5	3·43	—
6	1·49	—
7	1·13	—
8	1·16	—
9	0·71	—
10	0·21	—
12	0·08	—
14	0	—

Five-day-old SCF-grown tissue was subcultured to SCF medium either containing or lacking 5×10^{-7} M zeatin. At daily intervals thereafter, some of the tissue was fixed, Feulgen squashes prepared, and the mitotic index determined (mitotic index = (number of cells in mitosis)/ (total number of cells) × 100).

more than doubled after 1 day in culture, and it remained relatively high through the first 8 days of the culture period. Between the 8th and the 14th day of the culture period the mitotic index dropped to zero.

Polyribosome isolation

Sucrose density gradient analysis of extracts from 1-day-old soybean callus tissue maintained on SCF medium supplemented with zeatin exhibited a large fraction of the ribosomes (75%) complexed as polysomes (Fig. 1 A). However, if diethyl pyrocarbonate (DEP) was omitted from the isolation medium the amount of ribosomes

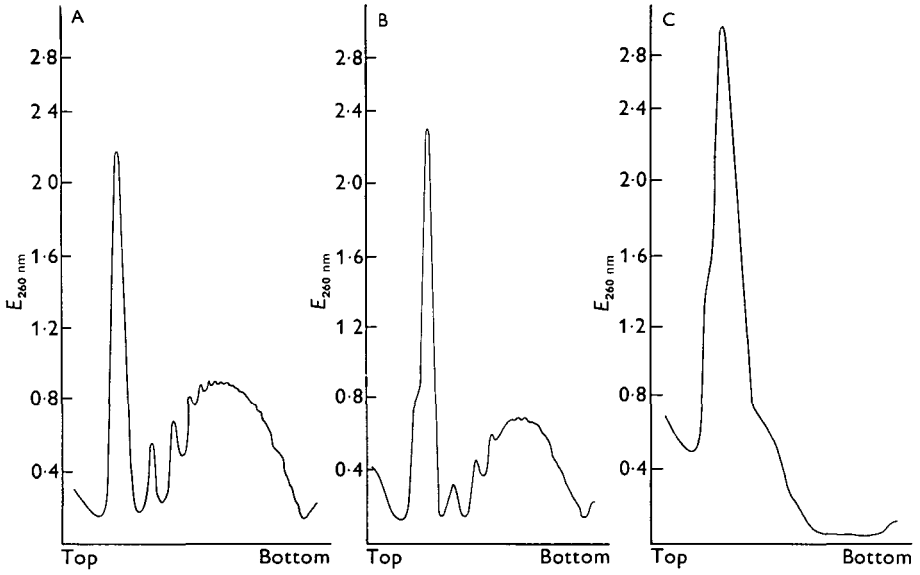

Fig. 1. A comparison of polyribosomal profiles obtained in the presence and absence of DEP and also in the presence of RNase. Ribosomal extracts from 1-day-old soybean tissue cultured on cytokinin-containing SCF medium were analysed by sucrose density gradient centrifugation. The profiles represent ribosomal material isolated from 2·0 g fresh weight of tissue. A, polyribosomes isolated in an extraction medium containing 0·1% diethyl pyrocarbonate (DEP) (Weeks & Marcus, 1969). B, polyribosomes isolated as in A, but without DEP in the extraction medium. C, the ribosomal extract was treated with 20 μg/ml pancreatic RNase for 30 min at 4 °C before sucrose density gradient centrifugation.

aggregated as polysomes was only 60% (Fig. 1 B). Furthermore, the gradient (Fig. 1 B) showed signs of degradation with a significant number of ribosomal subunits preceding the large monoribosome peak. Therefore DEP was added to the extraction medium to minimize the degradation of polyribosomes by ribonuclease.

In order to demonstrate that the u.v.-absorbing material observed in the polyribosome region of the gradient actually represented polyribosomes, the resuspended microsomal pellet was treated with 20 μg/ml of pancreatic ribonuclease (Calbiochem) at 4 °C for 30 min, and then fractionated on a sucrose gradient as described above.

As a result of the ribonuclease treatment, essentially all of the u.v.-absorbing material in the polyribosome region of the gradient was eliminated, and the height of the monoribosome peak was increased. This is consistent with the destruction by ribonuclease of the mRNA which held the ribosomal aggregation together to form the polyribosomes.

Cytokinin and polyribosome formation

The changes in the percentage of ribosomes bound as polyribosomes in soybean callus tissue cultured on SCF medium containing or lacking the cytokinin zeatin were determined. Three-week-old soybean was used as the inoculum and the percentage

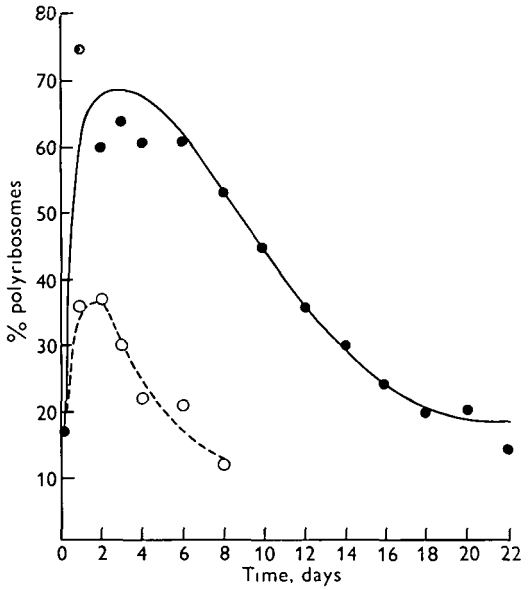

Fig. 2. Changes in the polyribosome content of soybean cells over the course of the culture period on medium containing or lacking cytokinin. Ribosomal extracts were obtained from soybean cells at intervals after culture on medium containing (—●—) or lacking (—○—) 5×10^{-7} M zeatin. The ribosomal material was analysed by sucrose density gradient centrifugation and the percentage of ribosomes present as polyribosomes determined by comparing the area under the monoribosome and polyribosome portion of the polysome profiles.

of polyribosomes found in these tissues was taken as the time 0 value. Samples were harvested at intervals throughout a subsequent 21-day culture period for the determination of polyribosome levels. As is shown by the data presented in Fig. 2, the cytokinin-grown tissues exhibited approximately a 4·5-fold increase in polyribosome content within 24 h of transfer to the zeatin-containing medium. After attaining this peak value, the polyribosome content declined to a high plateau level (about 3·7 times that of day 0) which was maintained until day 6. On day 8, the polyribosome content began a slow decline which continued throughout the remainder of the culture period.

Tissue which was subcultured on to medium lacking zeatin also showed an increased

polyribosome content within 24 h after transfer to freshly prepared medium. However, the extent of this increase was not as great as that observed in the cytokinin-treated tissues, amounting to approximately a 2-fold increase over the day 0 value. During the remainder of the 8-day culture period the polysome content of the hormone-deficient cells declined so that on the 8th day this tissue contained fewer ribosomes bound as polysomes than the inoculum.

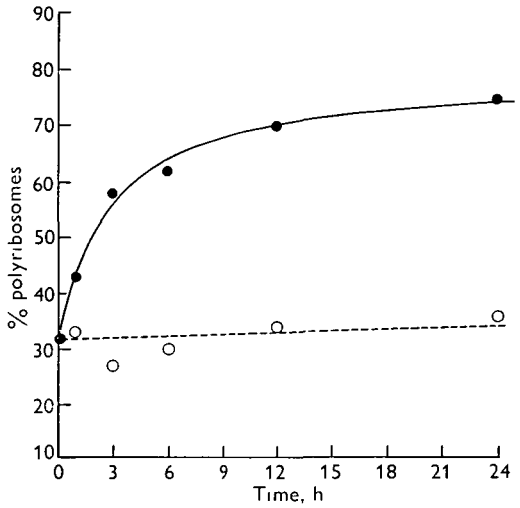

Fig. 3. The time course of cytokinin-induced polyribosome formation. Three-week-old soybean tissue was cultured for 24 h on SCF agar medium lacking cytokinin. The cells subsequently were transferred to SCF medium either containing (—●—) or lacking (—○—) 5×10^{-7} M zeatin. At intervals over the next 24 h, ribosomal extracts were prepared and analysed by sucrose density gradient centrifugation from aliquots of these cells.

The data in Fig. 2 demonstrate that there was a significant increase in polysome content of soybean cells within 1 day of transfer to SCF medium either containing or lacking cytokinin. An experiment was carried out to determine the degree to which cytokinin was involved in the enhanced polyribosome formation and to characterize the kinetics of cytokinin-induced polysome formation. To minimize the effect of any 'carry-over' of cytokinin from the previous culture medium, 21-day-old callus tissue was subcultured on to medium lacking cytokinin for 24 h. The tissue was then transferred to SCF medium either lacking or containing zeatin, and polyribosome analyses were made at frequent intervals.

The results of this experiment demonstrate that there was very little change in the polysome content of the soybean cells cultured in the absence of zeatin over the subsequent 24 h (Fig. 3). In contrast, soybean tissues transferred to zeatin-containing medium showed a 34% increase in polysome content within 1 h of transfer. The polysome content of these tissues continued to increase throughout the experimental period. Six hours after transfer it was almost double, and at 24 h it was 2·3 times that found at time 0.

The effect of actinomycin D on cytokinin-induced polyribosome formation

Since there was a very rapid increase in the polyribosome content of tissues following cytokinin treatment, the question arises whether the polysomes were formed with existing ribosomes and mRNA, or whether they represented newly formed protein-synthetic capacity. This was investigated as follows: Mature soybean callus tissues were subcultured to SCF liquid medium lacking cytokinin for a 24-h period. The explants were subsequently transferred to SCF liquid medium containing 5×10^{-7} M zeatin which was either devoid of or supplemented with actinomycin D (20 μg/ml).

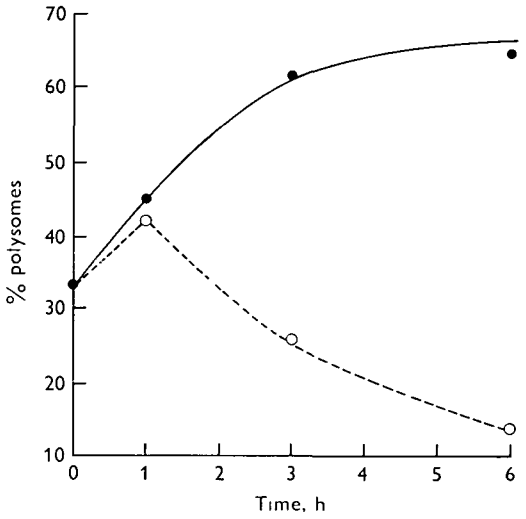

Fig. 4. Cytokinin-induced polyribosome formation in the presence of actinomycin D. Soybean cells first were cultured for 24 h in SCF liquid medium without cytokinin. Then the cells were transferred to SCF liquid medium supplemented with 5×10^{-7} M zeatin which either contained (—○—) or lacked (—●—) actinomycin D at 20 μg/ml. An analysis of the polyribosome content of the cells was carried out at intervals over the subsequent 6 h.

The cultures were maintained in darkness and polysome analyses were made over a 6-h period. The results in Fig. 4 indicate that in the presence of zeatin and absence of actinomycin D there was a rapid and continual increase in polyribosome content throughout the experimental period, whereas in tissue cultured in the presence of both zeatin and actinomycin D there was a 27% increase in polysomes within the first hour of the experimental period. However, after this time there was a gradual decline in polysome content so that after 3 and 6 h only 20% and 14% respectively of ribosomes were found as polysomes.

It was possible that the failure of actinomycin D to inhibit the cytokinin-induced increase in polysomes that occurred 1 h after hormone treatment was a result of the slow penetration of the inhibitor into the cells. Therefore, we added actinomycin D to zeatin-treated cells after the major portion of the cytokinin-induced increase in polysomes had occurred to see how rapidly actinomycin D brought about a reduction

in the level of polyribosomes. The results, shown in Table 2, indicate that actinomycin D treatment brought about a significant reduction in polysome levels within 1 h after the addition of the inhibitor. There was a progressive reduction in polysomes over the 6-h experimental period. The kinetics of this decay produced a curve that could be extrapolated back to the time of the addition of the drug.

Table 2. *Effect of actinomycin D on polyribosome levels in soybean callus*

Treatment	% polyribosomes
Control	52
Actinomycin D 1 h	42
3 h	37
6 h	33

Mature 21-day-old tissue was cultured in SCF liquid medium containing 5×10^{-7} M zeatin for 12 h, then transferred to the same medium containing actinomycin D (20 μg/ml). Polyribosome analyses were carried out at 1, 3, 6 h. Control tissue was that at time zero.

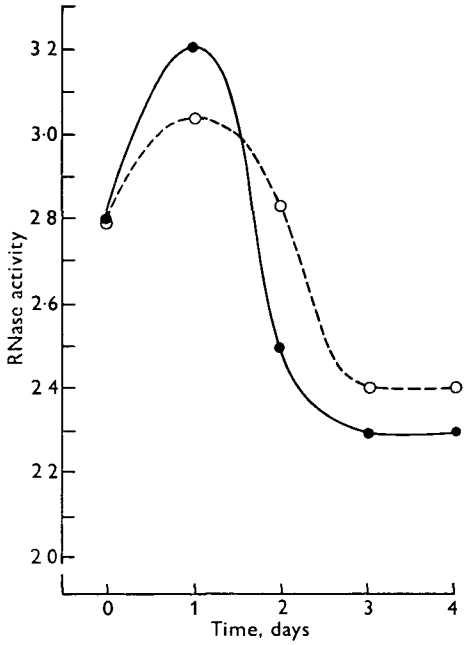

Fig. 5. Ribonuclease activity (μg RNA hydrolysed/mg protein) in soybean cells cultured on SCF medium in the presence and absence of cytokinin. Soybean cells were cultured in the presence (—●—) or absence (—○—) of 5×10^{-7} M zeatin. At daily intervals over the next 4 days some of the cells were sampled for determination of ribonuclease activity.

Ribonuclease activity

It has been suggested that cytokinins are able to regulate ribonuclease activity in plants (Srivastava, 1968). Although our polyribosome profiles show no obvious signs of degradation, we cannot completely rule out the possibility that the differences in

polyribosome content in cytokinin-treated and control tissues might result from a differential degradation of polysomes by ribonuclease during polysome isolation. Therefore, it was desirable to examine the ribonuclease activity of soybean tissues cultured in the presence and absence of cytokinin. Fig. 5 shows that on the 1st day after transfer, RNase activity was slightly higher in the tissues cultured on cytokinin-containing medium. However, during the remainder of the experimental period, RNase activity was slightly greater in tissues cultured on the medium lacking zeatin. The differences in RNase activity in cytokinin-grown and control tissue were not great at any time during the first 4 days of the culture period.

DISCUSSION

Part of the 4·5-fold increase in the polyribosome content of soybean cells observed 24 h after they have been subcultured to fresh medium containing zeatin was simply a transfer effect since tissues subcultured to medium lacking cytokinin showed a similar, though much smaller, increase. We were able to demonstrate that cytokinin treatment alone was capable of producing a dramatic increase in the percentage of ribosomes bound as polyribosomes by first preconditioning the cells in fresh medium lacking a cytokinin. There was an approximate 2-fold increase in polysomes during the 24 h preconditioning period, but no further increase in polysomes occurred when the tissue was transferred a second time to fresh medium lacking a cytokinin. However, if the preconditioned tissue was transferred to medium containing zeatin, the polyribosome content more than doubled. Furthermore, the bulk of this cytokinin-induced stimulation of polysomes occurred within 6 h after cytokinin treatment. Thus, depending upon the physiological state of the cells, cytokinin treatment can evoke a rapid and specific increase in the percentage of ribosomes bound as polyribosomes in cultured soybean tissues.

Hormone treatment of target cells has been shown to stimulate polysome formation in a variety of plant and animal systems. For example, diethylstilboesterol has been shown to stimulate polysome formation and protein synthesis in isolated chick oviduct (Means, Abrass & O'Malley, 1971). While the magnitude of this increase is similar to that elicited by cytokinin-treatment of soybean cells, the response occurs over a period of several days. Evins (1971) reported that the treatment of barley aleurone layers with gibberellic acid stimulated polysome formation, but only after a 3–4 h lag period. In contrast, we have found that cytokinin stimulates polyribosome formation in soybean cells with no lag detectable by the methods we have employed. If there is a lag period, it is considerably less than 1 h. Furthermore, the cytokinin-induced increase in polyribosomes even occurred in the presence of actinomycin D.

Preliminary results from our laboratory have indicated that cytokinin also is necessary for the maintenance of a high level of polysomes in the cultured soybean cells. When the cells are transferred to a medium lacking cytokinin, there is a rapid decay of polyribosomes, the kinetics of which appear to be the reverse of the cytokinin-induced promotion of polysome formation.

It is unlikely that ribonuclease activity could be invoked to explain the role of

cytokinin in either the promotion or the decay of polysomes in cultured soybean cells. As shown by the data in Fig. 5, cytokinin had only a slight effect on the ribonuclease activity of the soybean cells. Furthermore, ribonuclease digestion of polyribosomes produces a characteristic polysome profile which is considerably different from the polysome profiles we have obtained from soybean, except when exogenous RNase was added to the ribosomal pellets before sucrose density gradient centrifugation.

Cytokinins may regulate polyribosome formation at the translational level. The rapid decay of polysomes in cells treated with actinomycin D suggests that the average half-life of mRNA in these cells is something on the order of 3·5 h. This short half-life would argue against any significant translational control of metabolic and cytological processes. However, Murphy & Attardi (1973) recently have shown that the mRNA molecules in rapidly growing HeLa cells, which appear to have a half-life of 3–4 h as judged by polysome decay in the presence of actinomycin D, actually have a half-life of 2–3 days. Apparently actinomycin D not only blocks RNA synthesis, but it destabilizes existing mRNA as well. Assuming that soybean cells contain a pool of cytoplasmic, non-polysomal mRNA with a substantial half-life, cytokinins could stimulate polysome formation in the absence of additional RNA synthesis by modifying existing, inactive monoribosomes so that they could complex with cytoplasmic mRNA particles and reinitiate protein synthesis.

Cytokinins are able to modify ribosomes. Ralph, McCombs, Tener & Wojcik (1972) reported that cytokinin inhibited a protein kinase which phosphorylated Chinese cabbage leaf ribosomal proteins. We have found that cytokinins act in a similar manner in soybean cells (Tepfer, Short & Fosket, manuscript in preparation). While the role of ribosomal protein phosphorylation in polysome formation and protein synthesis is not known, Kabat (1972) has shown that monoribosomes have a different pattern of protein phosphorylation than polyribosomes in reticulocytes. There is a possibility that highly phosphorylated monoribosomes may represent storage ribosomes which are unable to reinitiate protein synthesis. By inhibiting ribosomal protein phosphorylation, cytokinins may prevent the formation of storage ribosomes and thus increase the percentage of ribosomes that are present in polyribosomes and engaged in protein synthesis.

A number of studies have shown that protein synthesis is required for the progression of cells through their division cycle (Stern & Hotta, 1963; Cummins & Rusch, 1966; Jones *et al.* 1968; Kim, Gebhard & Perez, 1968; Cummins, 1969; Parchman & Stern, 1969; Petersen, Tobey & Anderson, 1969; Rose, 1970; Everhart & Prescott, 1972; Highfield & Dewey, 1972; Webster & Van't Hof, 1973). However, all stages of the cell cycle are not equally dependent upon protein synthesis for their completion. For example, protein synthesis plays a particularly critical role in the initiation of DNA synthesis (Kim *et al.* 1968; Everhart & Prescott, 1972; Highfield & Dewey, 1972) while it does not appear to be required for mitosis itself, or for the events of late G_2 in animal cells (Petersen *et al.* 1969). In plants, however, the transition from G_2 to mitosis, and possibly even mitosis itself, is strongly dependent upon simultaneous protein synthesis (Rose, 1970; Webster, 1973).

The nature of the relationship between protein synthesis and progress through the

cell cycle is not at all clear. There is little doubt that the division cycle is ultimately under genetic control. Hartwell and his group have isolated a number of temperature-sensitive mutants of yeast which are blocked at specific points in their division cycle when they are placed at the restrictive temperature (Hartwell, Culotte & Reid, 1970; Hartwell, 1971*a*, *b*). Also, there is evidence for qualitative differences in the protein synthesized during different stages of the cell cycle (Kolodny & Gross, 1969; Fox & Pardee, 1971; Jouanneau, 1970). At least some of these stage-specific proteins are nuclear proteins (Jockusch, Brown & Rusch, 1970; Salas & Green, 1971) which may be involved in regulation.

However, none of this evidence requires the regulation of the mitotic cycle at the transcriptional level. We would like to propose that cytokinin allows G_2-arrested cells to enter mitosis by enhancing the rate of protein synthesis through some specific translational effect, and that this enhanced rate of protein synthesis allows the concentration of certain regulatory protein to reach the critical level to initiate chromosome condensation or spindle formation.

REFERENCES

ATKIN, R. K. & SRIVASTAVA, B. I. S. (1970). Studies on protein synthesis by senescing and kinetin-treated barley leaves. *Physiol. Plant.* **23**, 304–315.

BIRMINGHAM, B. C. & MACLACHLAN, G. A. (1972). Generation and suppression of microsomal ribonuclease activity after treatments with auxin and cytokinin. *Plant Physiol., Lancaster* **49**, 371–375.

CUMMINS, J. E. (1969). Nuclear DNA replication and transcription during the cell cycle of *Physarum*. In *The Cell Cycle. Gene-Enzyme Interactions* (ed. G. M. Padilla, G. L. Whitson & I. L. Cameron), pp. 141–158. New York: Academic Press.

CUMMINS, J. E. & RUSCH, H. P. (1966). Limited DNA synthesis in the absence of protein synthesis in *Physarum polycephalum*. *J. Cell Biol.* **31**, 577–583.

DAS, N. K., PATAU, K. & SKOOG, F. (1956). Initiation of mitosis and cell division by kinetin and indoleacetic acid in excised tobacco pith tissue. *Physiol. Plant.* **9**, 640–651.

DAS, N. K., PATAU, K. & SKOOG, F. (1958). Autoradiographic and microspectrophotometric studies of DNA synthesis in excised tobacco pith tissue. *Chromosoma* **9**, 606–617.

EVERHART, L. P. & PRESCOTT, D. M. (1972). Reversible arrest of Chinese hamster cells in G1 by partial deprivation of leucine. *Expl Cell Res.* **75**, 170–174.

EVINS, W. H. (1971). Enhancement of polyribosome formation and induction of tryptophane-rich proteins by gibberellic acid. *Biochemistry, N.Y.* **10**, 4295–4303.

FOSKET, D. E. & SHORT, K. C. (1973). The role of cytokinin in the regulation of growth, DNA synthesis and cell proliferation in cultured soybean tissues (*Glycine max* var. Biloxi). *Physiol. Plant.* **28**, 14–23.

FOSKET, D. E. & TORREY, J. G. (1969). Hormonal control of cell proliferation and xylem differentiation in cultured tissues of *Glycine max* var. Biloxi. *Plant Physiol., Lancaster* **44**, 871–880.

FOX, T. O. & PARDEE, A. B. (1971). Proteins made in the mammalian cell cycle. *J. biol. Chem.* **246**, 6159–6165.

HARTWELL, L. H. (1971*a*). Genetic control of the cell division cycle in yeast. II. Genes controlling DNA replication and its initiation. *J. molec. Biol.* **59**, 183–194.

HARTWELL, L. H. (1971*b*). Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Expl Cell Res.* **69**, 265–276.

HARTWELL, L. H., CULOTTE, J. & REID, B. (1970). Genetic control of the cell division cycle in yeast. I. Detection of mutants. *Proc. natn. Acad. Sci. U.S.A.* **66**, 352–359.

HIGHFIELD, D. P. & DEWEY, W. C. (1972). Inhibition of DNA synthesis in synchronized Chinese hamster cells treated in G1 or early S phase with cycloheximide or puromycin. *Expl Cell Res.* **75**, 314–320.

JOCKUSCH, B. M., BROWN, D. F. & RUSCH, H. P. (1970). Synthesis of a nuclear protein in G2-phase. *Biochem. biophys. Res. Commun.* **38**, 279–283.

JONES, R. F., KATES, J. R. & KELLER, S. S. (1968). Protein turnover and macromolecular synthesis during growth and gametic differentiation in *Chlamydomonas reinhardtii*. *Biochim. biophys. Acta* **157**, 589–598.

JOUANNEAU, J. P. (1970). Renouvellement des protéines et effet spécifique de la kinétine sur des cultures de cellules de tabac. *Physiol. Plant.* **23**, 232–244.

JOUANNEAU, J. P. & TANDEAU DE MARSAC, N. (1973). Stepwise effects of cytokinin activity and DNA synthesis upon mitotic cycle events in partially synchronized tobacco cells. *Expl Cell Res.* **77**, 167–174.

KABAT, D. (1972). Turnover of phosphoryl groups in reticulocyte ribosomal phospho-proteins. *J. biol. Chem.* **247**, 5338–5344.

KIM, J. H., GEBHARD, A. S. & PEREZ, A. G. (1968). Inhibition of DNA synthesis by actinomycin D and cycloheximide in synchronized HeLa cells. *Expl Cell Res.* **53**, 478–487.

KOLODNY, G. M. & GROSS, P. R. (1969). Changes in patterns of protein synthesis during the mammalian cell cycle. *Expl Cell Res.* **56**, 117–121.

LETHAM, D. S. (1967). Regulators of cell division in plant tissue. V. A comparison of the activities of zeatin and other cytokinins in five bioassays. *Planta* **74**, 228–242.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, N. J. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–273.

LYNDON, R. F. (1966). Intracellular distribution of ribonuclease in pea roots. *Biochim. biophys. Acta* **113**, 110–119.

MEANS, A. R., ABRASS, I. B. & O'MALLEY, B. W. (1971). Protein biosynthesis on chick oviduct polyribosomes. I. Changes during estrogen-mediated tissue differentiation. *Biochemistry, N.Y.* **10**, 1561–1570.

MILLER, C. O. (1961). A kinetin-like compound in maize. *Proc. natn. Acad. Sci. U.S.A.* **47**, 170–174.

MILLER, C. O., SKOOG, F., VON SALTZA, M. H. & STRONG, F. M. (1955). Kinetin, a cell division factor from deoxyribonucleic acid. *J. Am. chem. Soc.* **77**, 1392.

MURPHY, W. & ATTARDI, G. (1973). Stability of cytoplasmic messenger RNA in HeLa cells. *Proc. natn. Acad. Sci. U.S.A.* **70**, 115–119.

PARCHMAN, L. G. & STERN, H. (1969). The inhibition of protein synthesis in meiotic cells and its effect on chromosome behavior. *Chromosoma* **26**, 298–311.

PATAU, K. & DAS, D. K. (1961). The regulation of DNA synthesis and mitosis in tobacco pith tissue cultured *in vitro*. *Chromosoma* **11**, 553–572.

PAYNE, E. S., BROWNRIGG, A., YARWOOD, A. & BOULTER, D. (1971). Changing protein synthetic machinery during development of seeds of *Vicia faba*. *Phytochemistry* **10**, 2299–2303.

PETERSEN, D. F., TOBEY, R. A. & ANDERSON, E. C. (1969). Essential biosynthetic activities in synchronized mammalian cells. In *The Cell Cycle. Gene-Enzyme Interactions* (ed. G. W. Padilla, G. L. Whitson & I. L. Cameron), pp. 341–359. New York: Academic Press.

RALPH, R. K., MCCOMBS, P. J. A., TENER, G. & WOJCIK, S. J. (1972). Evidence for modification of protein phosphorylation by cytokinin. *Biochem. J.* **130**, 901–911.

ROSE, R. J. (1970). The effect of cycloheximide on cell division in partially synchronized plant cells. *Aust. J. biol. Sci.* **23**, 573–583.

SALAS, J. & GREEN, H. (1971). Proteins binding to DNA and their relation to growth in cultured mammalian cells. *Nature, New Biol.* **229**, 165–169.

SKOOG, F. & MILLER, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. exp. Biol.* **11**, 118–131.

SRIVASTAVA, B. I. S. (1968). Increase in chromatin associated nuclease of excised barley leaves during senescence and its suppression by kinetin. *Biochem. biophys. Res. Commun.* **32**, 533–538.

STERN, H. & HOTTA, Y. (1963). Facets of intracellular regulation of meiosis and mitosis. In Symp. int. Soc. Cell Biol. *Cell Growth and Cell Division* (ed. R. J. C. Harris), pp. 57–76. New York: Academic Press.

TORREY, J. G. (1961). Kinetin as trigger for mitosis in mature endomitotic plant cells. *Expl Cell Res.* **23**, 281–299.

TRAVIS, R. L. & KEY, J. L. (1971). Correlation between polyribosome level and the ability to induce nitrate reductase in dark-grown corn seedlings. *Plant Physiol., Lancaster* **48**, 617–620.

TREWAVAS, A. (1972). Control of the protein turnover rates in *Lemna minor*. *Plant Physiol., Lancaster* **49**, 47–51.

WEBSTER, P. L. (1973). Effects of cycloheximide on mitosis in *Vicia faba* root-meristem cells. *J. exp. Bot.* **24**, 239–244.

WEBSTER, P. L. & VAN'T HOF, J. (1973). Polyribosomes in proliferating and non-proliferating root meristem cells. *Am. J. Bot.* **60**, 117–121.

WEEKS, D. P. & MARCUS, A. (1969). Polyribosome isolation in the presence of diethyl pyrocarbonate. *Plant Physiol., Lancaster* **44**, 1291–1294.

WETTSTEIN, F. O., STAEHELIN, T. & NOLL, H. (1963). Ribosomal aggregate engaged in protein synthesis: Characterization of the ergosome. *Nature, Lond.* **197**, 430–435.

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