HORMONAL REGULATION OF GROWTH IN CULTURED PLANT CELLS

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SUMMARY

The group of naturally occurring plant hormones known as the cytokinins are defined by their ability to stimulate cell division in mature, differentiated, mitotically inactive cells in tissue culture. Evidence from the literature suggests that cytokinins play a specific role in regulating the progress of a plant cell through its division cycle; the hormone appears to trigger the transition from G2 to mitosis. However, the cytokinins are capable of evoking an array of physiological and developmental responses (many of which do not involve cell division) from different plant tissues and organs. One biochemical effect of the cytokinins is a dramatic and rapid stimulation of polyribosome formation in cultured soybean cells which require these hormones for growth. Stationary-phase soybean cells, transferred to a medium containing a cytokinin, double in cell number within 36 hr, but when transferred to a medium of the same composition lacking a cytokinin, they do not grow. In vivo labeling with [35S]methionine and slab-gel electrophoresis demonstrated that cytokinin brings about qualitative changes in the spectrum of proteins synthesized by soybean cells which precede hormone-induced cell division. We have shown that cytokinin-induced polyribosome formation is the result of an effect of the hormone on protein synthesis at the translational level. We propose that, in the absence of the hormone, certain genes are transcribed but their messengers are not translated. These include mRNA’s for specific cell division proteins. Cytokinins act as permissive factors, allowing cells to complete a genetically programmed sequence of events which was initiated by other factors.

Key words: cytokinin; cell division; polyribosome formation; translational control of protein synthesis.

INTRODUCTION

The modern work on the chemical regulation of plant cell division can be traced to the observation of van Overbeek, Conklin and Blakeslee (1) that coconut milk, the liquid endosperm of the coconut fruit, would support the growth of immature embryos. Coconut milk subsequently was employed to initiate growth in culture from a variety of differentiated, mitotically inactive tissues (2-4), while numerous attempts were made to identify the components of this substance that accounted for its activity (5, 6). Miller et al. (7) isolated a powerful plant cell division factor from autoclaved herring sperm DNA which they identified as 6-furfurylaminopurine and named kinetin. Although kinetin itself has not been found in any higher plant, other 6-substituted amino purines with the ability to stimulate cell division have been isolated from various plant materials, including coconut milk (8-12). These potent growth regulators have been given the generic name cytokinins to avoid confusion with the kinins found in animal tissues. Operationally, the cytokinins are defined as substances that are capable of initiating and sustaining cell division in excised tobacco pith tissue when it is cultured on a chemically defined medium also containing an auxin (13). Most naturally occurring cytokinins are N6-substituted adenines, some of which are illustrated in Fig. 1. The cytokinin with the trivial name, zeatin (6-(4-hydroxy-3-methyl-trans-2-butenylamine) purine), has been found in a wide variety of plant tissues.
Zeatin (6-(4-hydroxy-3-methyl-2-transbutenylamino) purine), as well as its riboside and ribotide, occur as free cytokinins in higher plants, whereas the cis isomer of zeatin (structure not shown) is found in plant t-RNA. 2-iPA has been found in the t-RNA of species ranging from E. coli to man, including higher plants. In higher plants and certain fungi it also occurs as a free cytokinin as shown and as the base. [Modified from Skoog and Armstrong (16) and Hall (17).]

and organs where it occurs free as the base, riboside or ribotide, and in macromolecular form, as the cis isomer, in transfer RNA (8-10, 14).

Cytokinins interact with other growth factors to initiate or modify a wide range of developmental and physiological events in higher plants. These include senescence, cell elongation, morphogenesis, cell differentiation and many others [see Kende (15), Skoog and Armstrong (16), Hall (17), for reviews]. In this paper, we will confine our attention to their function in the control of cell division where, perhaps somewhat unexpectedly (given the diversity of responses elicited by these substances), they exert a specific and somewhat unique regulatory effect, namely, to control the transition from G2 to mitosis. We also will give the reader a somewhat personal account of a mechanism by which cytokinin may exert this control.

CYTOKININS AS REGULATORS OF CELL DIVISION

There is a correlation between the amount of extractable cytokinin in a plant tissue or organ and its mitotic activity. In general, actively dividing tissues contain comparatively high levels of cytokinins, whereas mature, nongrowing tissues contain relatively little of these growth substances. For example, growing root tips appear to be a particularly good source of extractable cytokinins (18); and Phillips and Torrey (19, 20) have presented indirect evidence that the quiescent center of the apical meristem represents the site of cytokinin synthesis in the root. Cytokinins are transported from the root into the shoot through the xylem (21), which may explain some aspects of root-shoot interaction (22, 23). Organs such as shoot apical meristems, leaves and developing fruits, which have been shown to contain cytokinins, may represent targets for root-derived cytokinins or alternative sites of cytokinin biosynthesis.

Most of the work showing that cytokinins play a unique and highly specific role in regulating the plant cell division cycle has come from studies of cells and tissues in culture. Here two fundamentally different cytokinin-induced responses can be distinguished: (a) the initiation of cell proliferation from mature, mitotically quiescent, differentiated cells such as the tobacco pith and pea root cortex; and (b) the maintenance of mitotic activity in growing cell populations. The “dedifferentiation” of tobacco pith cells has been shown to involve profound cytological changes which precede the initiation of cell division (24, 25). In cultured pea root cortical tissues, cytokinin initiates two rounds of DNA replication before polyploid mitotic figures are observed (26, 27). Here again, dramatic cytological changes are observed to precede cell division. Recently Shininger (personal communication) has shown that one of the first detectable biochemical changes in the cytokinin-treated pea root cortical tissue is an accelerated rate of RNA synthesis.

None of these observations demonstrate that cytokinin has any specific effect on the cell cycle. They simply may have induced growth which, in these cases, happened to be accompanied by cell division. However, cytokinins also are required to sustain cell proliferation in some cultured cells. Continuous cell lines have been isolated from tobacco pith and soybean cotyledons which have continued to require cytokinin for growth after several years of routine subculturing (28-31). Fig. 2 illustrates the growth response to zeatin of cells derived from the cotyledons of the soybean cultivar, Sodifuri. These cells were isolated in 1970 by Dr. David Bilderback, then at the University of Oregon. They have been maintained in our laboratory since 1971 on a simple, chemically defined medium which contains inorganic salts, sucrose, inositol, thiamine, an auxin and the cytokinin zeatin. They are subcultured at 21-day intervals. Virtually no increase in either cell number