ISSN 0233-7657. Биополимеры и клетка. 1999. Т. 15. № 5

The phytohormone-mediated action of the synthetic regulators on cell extension growth in higher plants

Victoria A. Tsygankova, Vladimir N. Zayets, Larisa A. Galkina[1], Yaroslav B. Blume

Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine
Acad. Zabolotnoho vul., 148, Kyiv, 252627, Ukraine
[1]Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine
Murmanska vul., 1, Kyiv, 252600, Ukraine

It was shown that the action of synthetic growth activators on growth and development of isolated «non-decapitated» embryonic axes was analogous to action on embryonic axes of the intact haricot bean seeds; for the 72-hours period size increase of the embryonic axes depended on incubative medium composition: distilled water < lutidine N-oxide = 6-methylthiouracil < (IAA). Growth and development of «decapitated» embryonic axes in the same media sharply differed from the growth of «non-decapitated» axes: distilled water > lutidine N-oxide = 6-methylthiouracil < (IAA). In the experiments with embryonic axes in both of which one cotyledon was preserved («monocotyledonous seedlings») size increase was identical to size increase of embryonic axes in intact haricot bean seeds, as in the case of «non-decapitated» and «decapitated» embryonic axes, and it also depended on the incubative medium composition: distilled water < lutidine N-oxide = 6-methylthiouracil < (IAA). Investigation of the fractional composition of new synthesized [^{14}C] impulse labelled proteins using the method of one-dimensional PAG-electrophoresis and gel fluorography approach showed that lutidine N-oxide intensifies the synthesis of all cellular proteins fractions with predominating increase of the synthesis of some high-molecular and low-molecular polypeptides, and 6-methylthiouracil intensifies the synthesis of only one polypeptide with molecular weight of 30 kDa. The data obtained are examined accordingly as evidence in favour of ideas, developing in our investigation about phytohormone-mediated action of the synthetic plant growth regulators and different-direction mechanisms growth activating action of lutidine N-oxide and 6-methylthiouracil. The scheme of phytohormone-mediated action of the synthetic regulators on cell extension growth in higher plants is proposed.

Introduction. In published works [1, 2] devoted to screening and peculiarity of the biological action of synthetic plants growth regulators we stated hypothesis about mediated action of the synthetic activators through endogenous pool of phytohormones. It is known that the prominent role in mechanisms of cells growth by extension (which is dominant in plants embryos on early period of seeds germination, etc. at the start of «dark phase») belongs both to auxin-dependent as well as gibberellin-dependent cell wall enzymes. In dicot plants (in which the xyloglucan sidechains are abundant components of hemicellulose matrix of cell wall) such enzyme is $1 \rightarrow 4$ β-endoglucanase [3, 4]. In this case the cell wall elongation is controlled by growth hormone auxin, which causes acidification of the cell wall, activation of endoglucanase activity and release of xyloglucan fragments, which are well known as nona (x9) and hepta (x7) saccharide repeats.

In monocot plants (which contain 5 % xyloglucan in their cell wall; while the arabinogalactan sidechains are abundant components of hemicellulose matrix of cell wall) this role is carried out by dextranase enzyme (α-1,6-D-glucan-6-glucanohydrolase) [5]. This enzyme is known to be an auxin-dependent and its function comes to following: dextranase breaks down arabinogalactan crosslinks of the hemicellulose microfibrillar matrix of the cell wall with the release of arabinose and glucose. The breaking of these crosslinks will impart the necessary plasticity to the wall for cell extension to occur passively under the influence of intracellular osmotic pressure, formed by the vacuole.

The conclusion about dependence of dextranase activity from auxin concentration in plant tissues was made on the base of data obtained about high activity of this enzyme in normally growing, auxin-riched oat coleoptiles and low activity of dextranase in «decapitated» (deprived of top growth zone) coleoptiles with low auxin content in them [5].

Parallel with above mentioned enzymes the xyloglucan endotransglucosylase (XET) recently was found to be an gibberellin-activated [6]. XET can internally cleave the glucan backbone of a crosslinking xyloglucan molecular and remodel or rearrange the cellulose/hemicellulose network in the wall to accommodate the insertion of new microfibrils and therefore permit or even drive extension [7]. XET activity correlates with gibberellic acid-induced, but not auxin-induced, growth of pea stems, with growing regions of maize roots, elongating carrot suspension cells, and with ripening kiwi fruit cells.

The methodical approach [5], based on apical dominant phenomenon existing at plants (etc. that top and bottom «dominant centers» or pole growth points operate plant growth and development) was adapted in our investigation to verify direct or mediated through phytohormones action of synthetic growth stimulators using the embryonic axis of haricot bean as a model. So, the aim of our investigation was to study: 1) the action of lutidine N-oxide and 6-methylthiouracil on growth and development of isolated both intact and «decapitated» haricot bean embryonic axes (it is known that its growth to be realized by hypocotyl enlargement in early post-embryogenesis only); 2) the peculiarity of protein synthesis in embryonic axis cells of haricot bean seeds at stimulated germination.

Materials and Methods. In our experiments haricot bean seeds (*Phaseolus vulgaris L.*) of the variety «Bielozernaya» were used. Isolated embryonic axes were incubated between layers of filter paper moistened by distilled water (control) or by growth activators: lutidine N-oxide (ivin) or by 6-methylthiouracil (methyur) in concentration 1 mg/l. Incubation was carried out in the dark, in thermostat at 26 °C during 72 h. For «feeding» embryonic axes the mixture containing amino acids of protein casein hydrolyzate and glucose at finally concentration in solution 0.002 % accordingly were added to probes in the end of 48 h.

In one case separated from cotyledones whole embryonic axes were taken for the experiment (etc. ones containing full set of primary organs — root, hypocotyl and leaf), in other case, — «decapitated», etc. with separated top growth zone (leaf), from which, by analogy with coleoptyles, the phytohormone entering into bottom part of embryo-hypocotyl to realize.

Labelling of proteins. Embryonic axes were incubated during 1 h at the presence [^{14}C]-proline (specific radioactivity — 9870 mBk/mM), whose concentration in the samples was 100 mBk/ml.

Extraction of embryonic axes proteins. The tissue samples were frozen in liquid nitrogen and powdered carefully in a china mortar at 20 °C, and then dissolved in 20 mM sodium phosphate extracting buffer, pH 6.8, consisting of 75 mM NaCl, 200 mM of dithiothreitol (DTT), 1 mM of phenylmethylsulfonylfluoride and 100 μg of cycloheximide (the ratio between triturated material and extracting buffer being 1 : 10). Homogenate was passed through the filter «Miracloth», filtrate was placed in preliminarily cooled centrifugal test-tubes and was centrifuged consecutively at 18000 *g* (15 min) and at 105000 *g* (1 h). The temperature in camera was 4 °C. To precipitate the soluble plasmatic proteins 4 volumes of acidified acetone (pH 4,0) to final concentration (80 %) were added to the samples. The denatured proteins were precipitated by centrifugation at 8000 *g* (15 min) and after this precipitate was twice washed out by 80 % — ethanol and dried by acetone.

Electrophoresis of proteins. Proteins were dissolved in 100 μl of the buffer containing 0.0625 M tris-HCl, pH 6.8, 1 % of SDS, 10 mM of DDT, 7 % of phycole (or sucrose) and 0.025 % of bromphenol dark blue; 15 μg solutions of proteins were placed on each gel stripe. Vertical electrophoresis was carried out in 5—20 % gradient acrylamide gel at ratio acrylamide : bisacrylamide — 14 : 1 in Laemmli buffer system [8] according to Smith method [9]. The gel was polymerized in a block 16,5 × 16,5 × 0,1 cm. Preelectrophoresis was realized at voltage 70 V before stain entering into separating gel. Then the electrophoresis was continued at 120 V during 14 h. After proteins separation gel strips were fixed by 10 % acetic acid and 40 % isopropanol (25 min) and stained by 0.25 % Coomassie brilliant blue solution R-250 in 10 % acetic acid and 20 % isopropanol (25 min). The gel was washed out by a few volumes of 7 % acetic acid with 5 % isopropanol and dried using a heating vacuum-dryer («LKB», Sweden).

Fluorography of gel proteins. For this aim gel was saturated with fluorescent reagent 2,5-diphenyloxazole and exposed with X-ray film during two months at −70 °C.

Results and Discussion. The results of peculiarities of growth isolated haricot bean embryonic axes at the present both synthetic regulators and auxin are shown on fig. 1. In the control experiment (at incubation of embryonic axes in distilled water

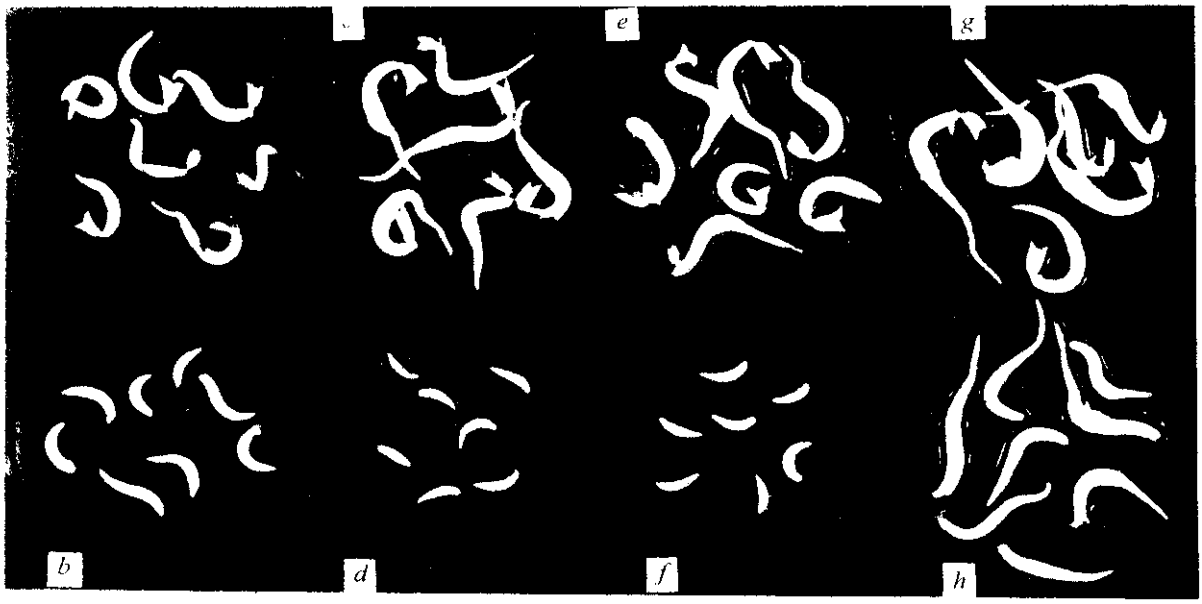

Fig. 1. The peculiarity of the growth of embryonic axes isolated from haricot bean seeds on different media: *a* and *b*; *c* and *d*; *e* and *f*; *g* and *h* — the growth «non-decapitated» and «decapitated» embryonic axes respectively (see consecutively from left to right): on distilled water and solutions of ivin, methyur and IAA

without any regulators during 72 h) the length of «non-decapitated» embryonic axes has been increased approximately in two times, while the growth of «decapitated» embryonic axes was perceptible lagging behind growth of native embryonic axes (fig. 1, *a*, *b*). At the same time, as well as in experiments with intact haricot bean seeds [1, 2], the growth of «non-decapitated» embryonic axes stimulated by ivin and methyur was sharply increased (fig. 1, *c*, *e*) in comparison with control. The contrary situation was observed with «decapitated» embryonic axes stimulated by synthetic growth regulators. In these experiments the growth of hypocotyles (unlike control growth) was inhibited completely, etc. their length remained on level of initial one (fig. 1, *d*, *f*) and what is more, after 72 h their autolysis began. Striking experiments were turned out with nature auxin (fig. 1, *g*, *h*). IAA sharply activated growth and development of isolated both «non-decapitated» and «decapitated» embryonic axes, and what's more, the growth of native embryonic axes exceed growth of ones in media with ivin and methyur, while growth of «decapitated» ones in medium with IAA was approximately same that «non-decapitated» in media with synthetic plant growth regulators.

As depicted in fig. 1 both under the action of synthetic growth regulators and auxin in peculiarity, not only elongation of embryonic axes size occurs during 72 h, but the development of root system from primary root begins. Consequently, the development of embryonic axes occurs normally in all cases (with the exception of ones, «decapitated» in media with synthetic growth regulators), but further growth of embryonic axes was discontinued in all variants of experiments owing to its separation from cotyledons from which, as is known, the main coming in plant embryos phytohormones (mainly gibberellins and cytokinins [10]) as well as nutrient substances occurs.

Therefore, these experiments synonymously have proved that synthetic plant growth regulators unlike exogenous auxin to act not direct, but exactly mediated through endogenous phytohormones way. Most likely, at early stages the increase of cell auxin concentration in top growth zone (primary leaf) and

after a while the phytohormones transport into embryonic axes from cotyledons of sprouting seeds stimulated by growth activators realize. The following fact testifies in this favour: in experiments with both «decapitated» and «non-decapitated» embryonic axes with preserved at them one cotyledons («monocotyledonous sprouts») an acceleration of growth and development of embryonic axes stimulated with both synthetic and nature regulators occurred as well as in the case of intact seeds.

It is evident that the acceleration of hypocotyl extension growth (stimulated with both synthetic and nature regulators) is to be caused first of all by indirect, mediated through phytohormones activation of the $1 \rightarrow 4$ β-endoglucanase (probably and XET) enzymes or by direct increase of its (their) catalytic activity or by increase of its (their) synthesis.

The results of investigations published recently [11, 12] confirm our hypothesis and prove that: 1) exogenous growth stimulators increase repeatedly concentration of endogenous pool of phytohormones; 2) ivin (lutidine N-oxide) possesses auxin-like activity (but, probably, also gibberellin-cytokinin activity).

At the same time Cooke et al. [13—15] have shown earlier that under the action 2,4-D the IAA synthesis in callus cells takes place on unusual means with use tryptophane as a precursor, moreover amount IAA reaches 600 ng/g of fresh weight, that vastly exceeds an usual level. When removing 2,4-D from nutrient medium the IAA concentration falls and reaches 15—30 ng/g of fresh weight, moreover its synthesis being realized through biochemical pathways, where tryptophane isn't to be used. As a rule, plants use themselves this way of IAA biosynthesis in natural conditions. Cooke et al. suppose that growth of callus cells are induced by synthetic analogue of auxin raising IAA concentration inside cell, with use tryptophane as a precursor. Short-term exposition plant explants at the presence extracellular synthetic auxin (2,4-D) can to induce proembryonic structure formation. According to ideas Cooke et al. when removing 2,4-D from nutrient medium occurs a recovering IAA synthesis to natural way, that, as a consequence, brings about transition of proembryonic structure into phase of somatic embryogenesis [15].

According to literary findings mentioned in J. Heyn's article [5], the processes of DNA, RNA and proteins synthesis are necessary «attribute» of cells growth by enlargement. It is really that increase of cell size in length, accompanied by formation a great number new cells from one cell by means of formation intracellular partitions simultaneously with duplication and transmission of gene material [7], as well as their differentiation occurring are attended with necessity to synthesize (in addition to preexisting) new structural and functional elements of different purposes: DNA or DNP, RNA and RNP, structural, functional and regulatory proteins, lipids, carbohydrates and others. And it's realized, probably, by following «scenario»: at first, most likely, amplification of ribosome genes takes place, then activation of rRNP and mRNP genes, synthesis of ribosome and mRNP-subparticles [16], tRNA and probably other types of RNA and further on chain — the synthesis of structural and other functional proteins of cell wall (which plays a central regulative role in both cells differentiation and specialization at early post-embryogenesis of embryo organism as well as at stalk's growth on following stages of plant development [17—22]); proteins for replication DNA as well as chromosome proteins, and proteins (enzymes) catalyzing synthesis of non-protein wall components.

For the aim to understand the peculiarity of proteins synthesis the fractional composition of new synthesized soluble proteins of embryonic axes cells in early period at stimulated and non-stimulated haricot bean seeds germination have been investigated comparatively in present work. The electrophoregram of separated summary stained and soluble cytozole proteins of embryonic axes cells of haricot bean seeds with stimulated and non stimulated germination are shown at the fig. 2, *A*, *a-c* (on the left). As it was to be expected, it was not possible to determine the fractions of new synthesized proteins (accounting 1—2 % proteins from all proteins mass) using one-dimensional PAGE against a background of the diversity of cellular proteins since new synthesized proteins being masked by preexisting major protein bands. In contrast to this, new synthesized proteins become selectively apparent at the fluorography of proteins of the same gel labelled before isolation by [^{14}C]-proline (fig. 2, *B*, *a'-c'*). One can see the increasing synthesis of all proteins fractions, synthesizing during ivin stimulation, although the synthesis of any high-molecular and low-molecular proteins fractions visibly predominates. However, methyur stimulates mainly synthesis of only one protein with molecular weight 30 kDa. As it was noted earlier [2], protein 30 kDa was discovered by us in abnormally high quantities in haricot bean seeds stimulated by methyur; total cell proteins were analyzed using two-dimensional gel-electrophoresis, and also by fluorography of synthesized [^{35}S]-labelled proteins in cell-free system from rabbit's reticulocytes using of poly(A)$^+$RNA as template RNAs, isolated from embryonic axes cells processed by methyur.

To date, reviewed by Showalter [21], five classes of proteins that are abundant in cell wall have been

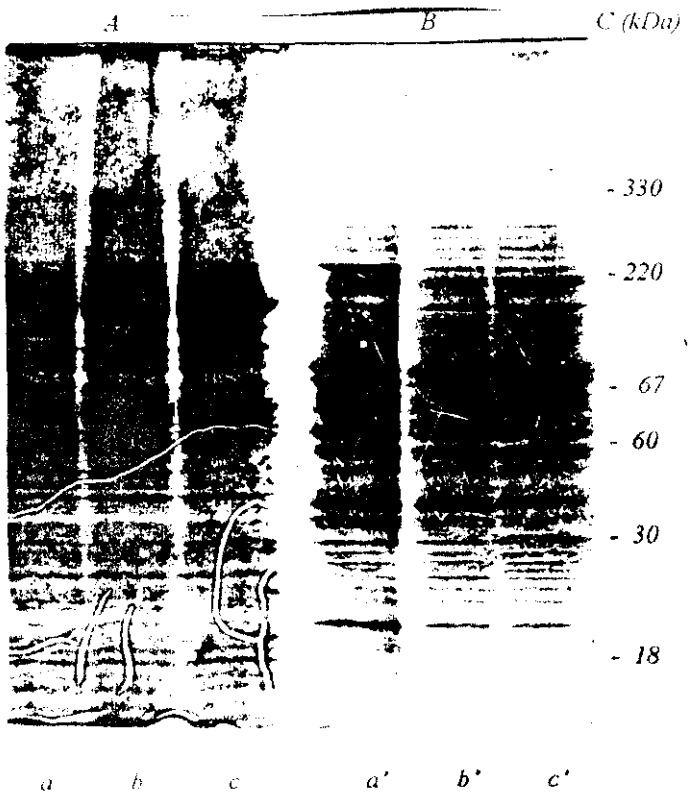

Fig. 2. PAGE-electrophoresis [^{14}C]-proline labelled *in vivo* summarized proteins of cell embryonic axes at stimulated and non-stimulated by growth regulators germination of haricot bean seeds: *A — fractional composition of proteins by stain; B* — gel fluorography of proteins; *C*—marker proteins: *a* and *a'* — protein fractions of embryonic axes of germinating haricot been seeds stimulated by ivin; *a* и *a'* — proteins from embryonic axes of haricot bean seeds with non-stimulated germination (control); *c* and *c'* — proteins from embryonic axes of germinating haricot bean seeds stimulated by methyur

studied in various plants. These are hydroxyproline-rich glycoproteins (HRGPs), glycine-rich proteins (GRPs), proline-rich proteins (PRPs), solanaceous lectins, and arabinogalactan proteins. All of this classes of proteins may be evolutionarily and functionally related to each other because they are enriched in hydroxyproline residues or share nucleotide sequence similarity. It is necessary to mention that these are not the only cell wall proteins that are known. Others exist, such as cysteine-rich thionins, 28- and 70-kD water-regulated proteins, a histidine-tryptophan-rich protein, and many cell wall enzymes such as peroxydases, phosphatases, invertases, α-mannosidases, β-mannosidases, β-1,3-glucanases, β-1,4-glucanases, polygalacturonase, pectin methylesterases, malate dehydrogenase, arabinosidases, α-galactosidases, β-galactosidases, β-glucuronosidases, β-xylosidases, proteases, and ascorbic acid oxidase and also above mentioned dextranase.

Extensins are a family of HRGPs and constitute the major protein components in cell walls of dicot plants (the molecular mass of glycoprotein is 86 kDa, while its polypeptide without carbohydrate part forms precisely 30 kDa) [18]. Extensins comprises 5—10 % from all proteins of cell wall and executes plural functions for cell, in particule take part in organization of carbohydrate carcass of primary cell wall and thus play essential role at cell extension growth. According with these works 30 kDa polypeptide is positively charged with high isoelectric point (pI of 9,9) due to a high content of Lys and/or His depending on the molecule, and soluble in water medium precursor of extensin. This polypeptide is synthesized and glycosylated by posttranslational modifications in cytoplasm and then integrated into the cell wall space. The expression one of the extensin gene family (SbHRGP3) increases with seedling maturation, and its expression is relatively high in the mature regions of the hypocotyl and in the root of soybean seedlings. Qi et al. [23] recently showed some evidence that extensins crosslink pectins in cell walls. Second, they contribute to plant defense, helping to protect mechanical wounding or against pathogen attack [22] (the latter probably results from positively charged extensins molecules interacting ionically with negatively charged surfaces of plant pathogens). As it was stated in our previous paper [2] the protein 30 kDa discovered by us and 30 kDa polypeptide of glycoprotein extensin are to be quite according to their physico-chemical characteristics. The determination of this protein is an important goal.

GRPs are characterized by their repetitive primary structure, which contains up to 70 % glycine arranged in short amino acid repeat units. There are at least two broad classes of GRPs. One class is found in cell wall and is developmentally regulated, whereas the second class is found somewhere in cytoplasm and is regulated by a variety of stress conditions, including abscisic asid and drought stress. Both GRP classes are represented in dicot and in monocot plants.

PRPs represent another relatively newly identified class of plant cell wall proteins of which at least some, and perhaps all, members contain hydroxyproline. There are at least two broad subclasses of PRPs: those that are components of normal plant cell

walls and those that are plant nodulins (i. e., proteins produced in response to infection by nitrogen-fixing bacteria) and constitute part of the nodule cell wall. Prolines as well as extensins take part in both growth and development regulation, cell wall structurization and counteract to stress factors.

Solanaceous lectins are specific ones for each plant species and appertain to chitin-uniting protein class. Solanaceous lectins consist of at least two distinct protein domains; one is rich in serine and hydroxyproline and contains the carbohydrate moiety, and the others are rich in glycine and cysteine. The serine-hydroxyproline-rich glycopeptide domain of Solanaceous lectins bears a striking biochemical resemblence to the extensins. Some previously proposed roles for the solanaceous lectins include cell wall structurization, sugar transport, stabilization of seed storage proteins, and control of cell division.

Arabinogalactan proteins (AGPs) are HRGPs that are generally very soluble and highly glycosylated. AGPs are widely distributed in plants and typically comprise only 2 to 10 % protein by weight. Their molecular weights are extremely heterogeneous, presumably reflecting different extents of glycosylation. The protein moiety of AGPs is typically rich in hydroxyproline, serine, alanine, threonine, and glycine and is resistant to proteolysis, a property that is presumably conferred by extensive glycosylation. AGPs have isoelectric points in the range of pH 2 to 5. AGPs have been proposed to act as glues, lubricants, and humectants. Their general abundance in the middle of the wall and in the styles of angiosperms and in the medulla of root nodules makes them likely candidates for functioning in cell-cell recognition. There is also some indication that AGPs accumulate in response to wounding, and a role for these AGPs in floral histogenesis and differentiation is possible.

Such ones are related to plasmalemma proteins, which functionate in cytoplasma and provide cell wall with structural and functional elements [4]: 1) cellulose synthase (β-1,4-glucansynthase)-prominent enzyme of cellulose synthesis (assembly) from preformed «blocks» (glucose molecules) — is localized and functionates (as other enzymes, participating in synthesis of cellulose precursors) in plasmalemma cells (in its thickness), although, according to some findings cellulose synthase has bimodal distribution (etc. is localized both in cell wall bordering with plasmalemma zone and in plasmalemma; 2) callose synthase is known to be a major polymer, produced by higher plant plasma membrane. This enzyme provide synthesis polysaccharide callose (1,3-β-glucans) [24—26].

The morphogenetically important elements that provide spatial information of newly synthesized cellulose microfibrils (MFs) and control their orientation are cortical microtubules (MTs) [27—30]. The base of MT are protein tubulin, which consist from alpha and beta subunits, existing in a 1 : 1 stoichiometry to one another [27, 28, 31]. The gamma-tubulin subunits are found too. The various tubulin isoforms may differentially modulate the function of MTs. There are several possible mechanisms by which cell might control the stability of cortical MTs. The most likely mechanisms are: 1) qualitative changes in tubulin gene expression [31]; 2) tyrosination/detyrosination or acetylation of alpha-tubulin [32—33]; 3) changes in MAP interactions [34]; 4) phosphorylation of MT-protein [27]; 5) fluctuations of calcium levels [35]. It was fond that cell wall protein extensin takes part in stabilization cortical MTs [36].

MT/plasma membrane (PM) associations are the main process of cell wall formation and, possible, can to realize by different means [27]: 1) MTs may associate directly with tubulin subunits in the PM [37]; 2) the association may be via integral or peripheral membrane proteins; 3) peripheral bridging protein associates with membrane tubulin within the hydrophobic milieu of PM, and with MTs in the hydrophilic environment of the cortical cytoplasm; 4) actin microfilaments also presented in the cortex can, in some instances, alter the organizational status of cortical MTs [38]. Cellulose is synthesized by a cellulose synthase complex, which forms membrane rosettes that are believed to be capable of moving in the plane of the membrane [39]. The MTs restrict this movement, thereby providing channels for the cellulose-synthesizing complex [40]. As a result, the order of newly deposited cellulose fibrils reflects that of the underlying cortical MTs. During cell elongation and differentiation MTs become more ordered into characteristic transverse arrangement [41, 42].

The adduced above list of proteins «spectrum» which are presented in cell wall and their any characteristics demonstrates clearly that structural and functional peculiarities of wall are determined by its proteins. But cellular, tissue and species specificity of wall not only depends on structural peculiarities and proteins set but also and correlation between protein and non-protein cell wall components. Among non-proteins components in wall, besides cellulose and hemicellulose there are also lignin, propectin, cutin, suberin and any other products of secondary synthesis [17—22]. It's evidently that study of biogenesis cell wall proteins (and other its components) as well as plasmalemma proteins «serving» cell wall at stimulated plant growth and development, peculiarly

in early postembryonic period (in the beginning of «dark phase») is highly perspective for determination action points of growth activators in molecular level.

In conclusion, on the grounds of results our investigations and literature analysis, we composed the scheme of the mechanisms of increase extension cells growth by synthetic phytohormone substitutes (fig. 3). Some different facts are reflected in it:

1) the well known data that cell wall before differentiation (that namely is able to enlargement) consists of one layer (primary-differentiated wall). It's main constituents are cellulose microfibrils, proteins and other substances, immersed into hemicellulose matrix (cellulose content in matrix is 30 %). After differentiation the second layer appears in cell wall, being, on the whole, cellulose fibrils, fit framing wall and penetrating it. The secondary, or differentiated wall is already not possible to extension (cellulose content in it with respect to general weight is 60 %);

2) the main processes of cell growth in length are enlargement and differentiation of cell wall;

3) the results obtained in our work witness hypothesis, that growth effects, caused by growth stimulators, depend on action of synthetic compounds on these processes.

So, we suggest that penetration of growth regulators inside cells occurs by one of two following possible ways: 1) through receptor cells system (but availability of receptors, carrying out such functions, is not proved severely for plants, especially in case of synthetic growth regulators); 2) it's most probably, the penetration of growth regulators is realized through the channels of ATP-dependent proton pompe (as it's shown, for example, in the case of negatively charged auxin [43]).

The synthetic compounds inside of cells increase active pool of endogenous phytohormones with growth stimulating activity at simultaneous taking off inhibitory action of ABK. In particular, increase of auxin concentration causes the increase of $1 \rightarrow 4$ β-endoglucanase activity (or another above mentioned enzymes with analogous action) which catalyses cell wall growth by enlargement. Under the action either phytohormones activated by growth stimulators, an amplification of ribosome genes, their expression and expression of genes, which code structural and functional cell wall proteins and plasmalemma proteins, «serving» cell wall (cellulose synthase, realizing cellulose synthesis, and tubulin protein, providing microtubules formation) are to be induced. The microtubules control the spatial orientation of newly synthesized cellulose microfibrils. Probably, severe coordination in time of processes enumerated exists,

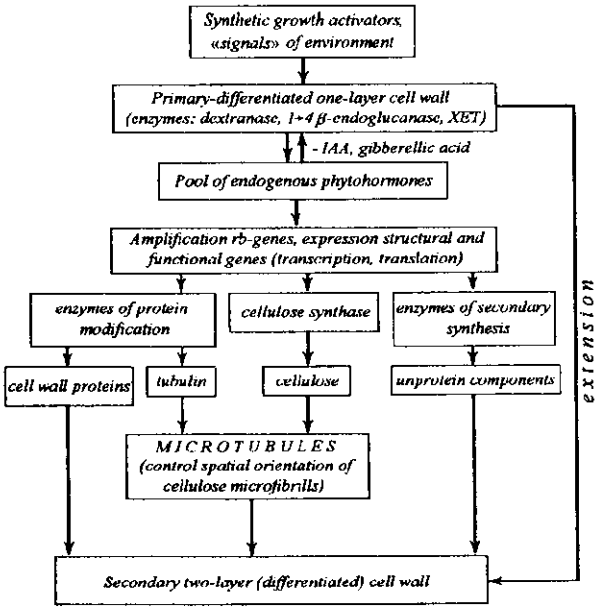

Fig. 3. Scheme of phytohormone-mediated action of the synthetic plant growth regulators

in which succession of synthesis and integration into cell wall of structural and functional elements is one of the main point.

However, the events taking place under action of growth stimulators with sharply different from phytohormones synthetic structure may be another. The pool of endogenous phytohormones being non-peculiar for either period of development, including selectively uncompleted cascade of genes, which control growth process, may be activated. As a result, the disharmony in natural ratio of cell wall components (first of all in set of its proteins) may to arise. Superfluous synthesis of any proteins and deficit of wall in other proteins may be reason for infringement of its differentiation (and cell on the whole) and, as a consequence, modification of plant ontogenesis. For example, the picture like this is observed following methyur action on sprouting haricot bean seeds (appearing in cells 30 kDa protein in large quantity; which is, probably, precursor of extenin glycoprotein, and its appearing causes shortening of the plant ontogenesis almost twice [2]). On the contrary, when sprouting haricot bean seeds were stimulated by lutidine N-oxide (that is accompanied by sharp increasing of all cell proteins synthesis with primary

synthesis of some ones) an intensified development of vegetative plant organs and delay in development of generative (reproductive) ones occur [2].

Probably, the plant dwarfism is caused by mutation of those genes which are related to wall proteins biogenesis. In this case, most probably, gene mutations, programming synthesis of phytohormones (which regulates activity of genes (gene), coding protein synthesis of the wall) take place. Restoration of growth to normal level (or even outstripping of normal growth) of dwarfish plants, treated by exogenous growth stimulators (auxin, gibberellins) point out on this [44].

Described in literature effects of plant stability to different diseases and stress factors of the environment obtained under action of synthetic regulators [45—47] are also conditioned, probably, by increase synthesis of any cell wall proteins, providing either forms of stability (the first protective barrier, the second — intracellular protective mechanisms). For example, increase stability to pathogens may be connected with rising the synthesis of glycoprotein extensin. Stability to other factors may be also provided by increase of synthesis both this protein and other cell wall proteins by synthetic growth activators on early stages of postembryogenesis.

One can not except also at artificially stimulated growth the possibility activation of plasmalemma proteins, completing cell wall with structural elements. It is possibly that synthetic growth regulators through phytohormone pool might qualitatively change tubulin gene expression or modify isotypes of tubulin subunits by above mentioned mechanisms [32, 34, 35], and as a consequence, increase stability MTs.

Therefore, it's evidently that study of biogenesis, structure and function of cell wall components and functional proteins of plasmalemma, participating in the cell wall formation, of the one hand; phytohormone pool activated by growth regulators as well as genes activated by phytohormones — on the other hand, during all period of plant ontogenesis will give possibility to determine the role of each cell wall protein and plasmalemma protein, «serving» the wall, in the processes of cell differentiation and specialization, and also to determine, which genes are responsible for these basic for plant cells processes.

В. А. Циганкова, В. Н. Заець, Л. А. Галкіна, Я. Б. Блюм

Опосередкована фітогормонами дія синтетичних регуляторів на ріст клітин розтягненням у вищих рослин

Резюме

Показано, що дія синтетичних активаторів росту на розвиток та ріст ізольованих «недекапітованих» зародкових осей була аналогічною за своїм характером до дії на зародкові осі інтактного насіння квасолі; за 72-годинний період збільшення розмірів зародкових осей залежало від складу середовища інкубації: дистильована вода < N-окис лутидину = 6-метилтіоурацил < ІОК. Ріст і розвиток «декапітованих» осей на тих самих середовищах різко відрізнявся від росту «недекапітованих»: дистильована вода > N-окис лутидину = 6-метилтіоурацил < ІОК. В експериментах із зародковими осями, у яких збережено по одній сім'ядолі («однодольні» кільчики), як у випадку з «недекапітованими», так і «декапітованими» зародковими осями збільшення розмірів було ідентичним такому зародкових осей інтактного насіння квасолі і також залежало від складу інкубаційного середовища: дистильована вода < N-окис лутидину = 6-метилтіоурацил < ІОК. Вивчення фракційного складу новосинтезованих (^{14}C]-мічених білків зародкових осей методом одномірного ПАГ-електрофорезу з наступною флюорографією гелів засвідчило, що N-окис лутидину підсилює синтез усіх фракцій клітинних білків з переважаючим збільшенням синтезу деяких високо- і низькомолекулярних поліпептидів, а 6-метилтіоурацил підсилює синтез тільки одного поліпептиду з молекулярною масою 30 кДа. Отримані дані можуть розглядатися відповідно як доказ висунутих нами положень стосовно фітогормон-опосередкованої дії синтетичних регуляторів росту рослин та різноспрямованості механізмів рістактивуючої дії N-окису лутидину та 6-метилтіоурацилу. Запропоновано схему опосередкованого фітогормонами механізму дії синтетичних регуляторів на ріст клітин розтягненням у вищих рослин.

В. А. Цыганкова, В. Н. Заец, Л. А. Галкина, Я. Б. Блюм

Опосредуемое фитогормонами действие синтетических регуляторов на рост клеток растяжением у высших растений

Резюме

Показано, что действие синтетических ростактиваторов на рост и развитие изолированных «недекапитированных» зародышевых осей было аналогичным по своему характеру действию на зародышевые оси интактных семян фасоли; за 72-часовой период увеличение размеров зародышевых осей зависело от состава среды инкубации зародышей: диситиллированная вода < N-окись лутидина = 6-метилтиоурацил < ИУК. Рост и развитие «декапитированных» зародышевых осей на тех же средах резко отличались от роста «недекапитированных» осей: дистиллированная вода > N-окись лутидина = 6-метилтиоурацил < ИУК. В опытах с зародышевыми осями, у которых было сохранено по одной семядоле («однодольные» проростки), как в случае с «недекапитированными», так и «декапитированными» зародышевыми осями увеличение размера было идентично таковому зародышевых осей в интактных семенах фасоли и также зависело от состава инкубационной среды: диситиллированная вода < N-окись лутидина = 6-метилтиоурацил < ИУК. Изучение фракционного состава новосинтезированных (^{14}C]-импульсно меченных) белков зародышевых осей методом одномерного ПАГ-электрофореза с последующей флюорографией гелей показало, что N-окись лутидина усиливает синтез всех фракций клеточных белков с преобладающим увеличением синтеза некоторых высоко- и низкомолекулярных полипептидов, а 6-метилтиоурацил усиливает синтез только одного полипептида с молекулярной массой 30 кДа. Полученные данные рассматриваются соответственно как доказательство в пользу развиваемых нами представлений о фитогормон-опосредуемом действии синтетических регуляторов роста растений и о разнонаправленности механизмов ростактивирующего действия N-окиси лутидина и 6-метилтиоурацила. Предложена схема опосредуемого фитогормонами механизма действия синтетических регуляторов на рост клеток растяжением у высших растений.

REFERENCES

1. *Tsygankova V. A., Blume Ya. B.* Screening and peculiarity of the biological action of synthetic plant growth regulators // Biopolimeri i kletka.—1997.—13, N 6.—P. 484—492. (In Engl.)
2. *Tsygankova V. A., Zayets V. N., Galkina L. A., Prikazchikova L. P., Blume Ya. B.* An unusual minor protein appearing in embryonic axis cells of haricot bean seeds following germination process stimulated by 6-methylthiouracyl // Biopolimeri i kletka.—1998.—14, N 5.—P. 438—448. (In Engl.)
3. *Farcas V., Hanna R., Maclachlan G.* Xyloglucan oligosaccharide α-L-fucosidase activity from growing pea stems and germinating *Nasturtium* seeds // Phytochemistry.—1991.—30.—P. 3203—3207.
4. *Levy S., Staehelin A.* Synthesis, assembly and function of plant cell wall macromolecules // Curr. Opin. Cell Biol.—1992.—4.—P. 856—862.
5. *Heyn A. N. J.* Molecular basis of auxin-regulated extension growth and role of dextranase // Proc. Nat. Acad. Sci. USA.—1981.—78, N 11.—P. 6608—6612.
6. *Poter I., Fry S. C.* Xyloglucan endotransglucosylase activity in pea internodes // Plant Physiol.—1993.—103.—P. 235—241.
7. *Keith R.* The plant extracellular matrix: in a new expansive mood // Curr. Opin. Cell Biol.—1994.—6.—P. 688—694.
8. *Laemmli U. K.* Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature.—1970.—227.—P. 680—685.
9. *Smith B. J.* SDS polyacrylamide gel electrophoresis of protein // Methods in molecular biology. Proteins / Ed. J. M. Walker.—Clifton; New Jersey: Humana press, 1984.—Vol. 1—P. 41—56.
10. *Varshavskaya V. B.* Ideas of N. G. Kholodniy about plant growth regulators in agricultural practice // Ukr. Bot. Jurn. (Kyiv).—1982.—39, N 3.—P. 87—96.
11. *Terek O., Romaniuk K. N., Terec K.* Endogenous phytohormones of plants treated by growth regulators // 2nd Conf. on progress in plant sciences from Plant Breeding to growth Regulation (15—17 June 1998, Mosonmagyarovar): Abstracts.—Bergholz-Rehbrucke, 1998.—P. 64.
12. *Romaniuk N., Troyan V., Musiyaka V., Bezveniuk Z., Terec O.* Investigation of growth regulation activity of Emistym, the new perspective plant growth regulator // 2nd Conf. on progress in plant sciences from Plant Breeding to growth Regulation (15—17 June 1998, Mosonmagyarovar): Abstracts.—Bergholz-Rehbrucke, 1998.—P. 75.
13. *Michalczuk L., Cooke T. J., Cochen J. D.* Auxin levels at different stages of carrot embryogenesis // Phytochemistry.—1992.—31.—P. 1097—1103.
14. *Michalczuk L., Ribnicky D. M., Cooke T. J., Coken J. D.* Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures // Fisiol. Rastenij (Russia).—1992.—100.—P. 1346—1553.
15. *Cooke T. J., Racusen R. H., Cohen J. D.* The role of auxin in plant embryogenesis // Plant Cell.—1993.—5.—P. 1494—1495.
16. *Kukhar V. P., Pushkariov V. M., Galkina L. A., Ponomarenko S. P., Galkin A. P.* Ivin-yan induced stimulation of cytoplasmic ribonucleoproteinic particle biosynthesis in embryonic axis cells from germinating haricot bean seeds // Dop. AN Ukraini Ser. B.—1987.—№ 7.—P. 76—78. (In Rus.)
17. *Wareing P. F., Phillips I. D. J.* Growth and differentiation in plants: Transl. from Engl.—Moscow: Mir, 1984.—512 p.
18. *Chen J., Varner J. E.* An extracellular matrix protein in plants: characterization of a genomic clone for carrot extensin // EMBO J.—1985.—4, N 9.—P. 2145—2151.
19. *York W. S., Darvill A. G., Neil M. M., Stevenson T. T., Albersheim P.* Isolation and characterization of plant cell walls and cell wall components // Meth. Enzymol.—1986.—P. 3—40.
20. *Evans I. M., Gatehouse L. N., Yarwood J. N., Boulter D., Croy R. D.* The extensin gene family in oilseed rape (*Brassica napus L.*): characterization of sequences of representative members of the family // Mol. and Gen. Genet.—1990.—223.—P. 273—287.
21. *Showalter A. M.* Structure and function of plant cell wall proteins // The Plant Cell.—1993.—5, N 1.—P. 9—23.
22. *Ahn J. H., Choi Y., Kwon Y. M., Kim S. G., Choi Y. D., Lee J. S.* A novel extensin gene encoding a hydroxyproline-rich glycoprotein requires sucrose for its wound-inducible expression in transgenic plants // The Plant Cell.—1996.—8.—P. 1477—1490.
23. *Qi X., Behrens B. X., West P. R., Mort A. J.* Solubilization and partial characterization of extensin fragments from cell walls of cotton suspension cultures: evidence for a covalent cross-link between extensin and pectin // Plant Physiol.—1995.—108.—P. 1691—1701.
24. *Delmer D. P.* Cellulose biosynthesis // Annu. Rev. Plant Physiol.—1987.—38.—P. 259—290.
25. *Delmer D. P., Solomon N., Read S. M.* Direct Photolabeling with [P^{32}] UDP-glucose for identification of a subunit of cotton fiber callose synthase // Plant Physiol.—1991.—95.—P. 556—563.
26. *Dhugga K. S., Ray P. M.* Isoelectric focusing of plant plasma membrane proteins // Plant Physiol.—1991.—95.—P. 1302—1305.
27. *Cyr R. J.* Microtubules in plant morfogenesis: role of the cortical array // Annu. Rev. Cell. Biol.—1994.—10.—P. 153—180.
28. *Goddard R. H., Wick S. W., Silflow C. D., Snustad D. P.* Microtubule components of the plant cell cytoskeleton // Plant Physiol.—1994.—104.—P. 1—6.
29. *Giddings T. H., Staehelin L. A.* Microtubule-mediated control of microfibril deposition: a re-examination of the hypothesis // The cytoskeletal basis of plant growth and form / Ed. C. W. Lloyd.—San Diego: Academic, 1991.—P. 85—100.
30. *Morejohn L. C.* The molecular pharmacology of plant tubulin and microtubules / Ed. C. W. Lloyd.—San Diego: Academic, 1991.—P. 29—44.
31. *Hussey P. J., Silflow C. D.* Tubulin gene expression in higher plants // Ed. C. W. Lloyd.—San Diego: Academic, 1991.—P. 15—28.
32. *Smertenko A., Blume Ya. B., Viklicky V., Opatrny Z., Draber P.* Posttranslational modifications and multiple isoforms of tubulin in *Nicotiana tabacum* cells // Planta.—1997.—201.—P. 349—358.
33. *Blume Ya., Smertenko A., Ostapets N. N., Viklicky V., Draber P.* Posttranslational modifications of plant tubulin // Cell Biol. Int.—1998.—21, N 12.—P. 918—920.
34. *Hotani H., Horio T.* Dynamics of microtubules visualised by darkfield microscopy: treadmilling and dynamic instability // Cell Motil. Cytoskeleton.—1988.—10.—P. 229—236.
35. *Cyr R. J.* Calcium/calmodulin affects microtubule stability in lysed protoplasts // J. Cell Sci.—1991.—100.—P. 311—317.
36. *Akashi T., Kavasaki S., Shibaoka H.* Stabilization of cortical microtubules by the cell wall in cultured tobacco cell // Planta.—1990.—182.—P. 363—369.
37. *Laporte K., Rossignol M., Traas J. A.* Interaction of tubulin with plasma membrane-tubulin is present in purified plasmalemma and behaves as integral membrane protein // Planta.—1993.—191.—P. 413—416.
38. *Staiger C. J., Yan M., Valenta R., Shaw P. J., Warn R. M.* Microinjected profilin affects cytoplasmic streaming in plant

cells by rapidly depolymerizing actin microfilaments // Curr. Biol.—1994.—4.—P. 215—219.
39. *Staehelin L. A., Giddings T. H.* Membrane-mediated control of cell wall microfibrillar order // Developmental order: its origin and regulation / Eds S. Subtelny, P. B. Green.—New York: Liss, 1982.—P. 133—147.
40. *Staehelin L. A., Giddings T. H.* Spatial relationship between microtubules and plasma-membrane rosettes during the deposition of primary wall microfibrils in *Closterium sp.* // Planta.—1988.—173.—P. 22—30.
41. *Laskowski M. J.* Microtubule orientation in pea stem cells: a change in orientation follows the initiation of growth rate decline // Planta.—1990.—181.—P. 44—52.
42. *Baluska F., Parker J. S., Barlow P. W.* Specific patterns of cortical and endoplasmic microtubules associated with cell growth and tissue differentiation in roots of maize (*Zea mays L.*) // J. Cell Sci.—1992.—103.—P. 191—200.
43. *Ranjeva R.* Signal transduction in plant growth and development // Plant Science.—1997.—126, N 2, 8.—P. 227—228.
44. Plant growth and it's regulation (genetics and physiological aspects) / Eds B. I. Kefely, S. I. Toma.—Kishinev: Shtiintsa, 1985.—P. 222.
45. *Plant* growth regulators / Eds V. S. Shevelucha, V. P. Kukhar, A. A. Sozinov.—Kyiv, 1992.—178 p.
46. *Plant* growth regulators in agricultural / Eds A. O. Shevchenko.—Kyiv, 1998.—143 p.
47. *Kornyeyev D. Yu., Kochubey S. M., Ponomarenko S. P., Borovikova G. S., Shevchenko O. V.* The increase of the maize seedlings tolerance to the high temperature stress upon treatment with plant growth regulator emistim C // II Int. Symp. on Plant Biotechnol. (4—8 October 1998): Abstracts.—Kyiv, 1998.—175 p.

УДК 631.811.98
Received 24.02.99