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CONCERNING PECULIARITIES OF GENE EXPRESSION CHANGES IN PLANT LEAF CELLS DURING TWENTY-FOUR-HOUR PERIOD

V. A. Tsygankova Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine, Kyiv

Using DOT-blot hybridization DNA with RNA, the analysis of degree homology of products of gene expression (cytoplasmic mRNAs) was fulfilled from the cells of dicotyledonous (soya, pea) and monocotyledonous plants (wheat, corn) leaves followed by their separation from plants in an early period with weak photosynthesis (at 5 a. m.), in a middle period (at 2 p. m.) with strong photosynthesis and in the evening period (at 9 p. m.) with weak photosynthesis towards mRNA from plant leaves being in a dark phase (at 2 a. m. — control). It was shown that divergence in mRNA populations between experience and control begins early in the morning at weak sunlight, increases sharply at daylighting (at 2 p. m.) and drew in at the sunset. Emistim C increases difference between control and 5-hour time-of-day to a level of 2-hour period. It is assumed that these changes occur by means of «windmill switching» the genes belonging to one family but having different activity due to their location under promoters of different force (promoters of weak, moderate and strong force) and such type of plasticity (maneuverability) of plant adaptation to the environment is achieved by such mechanism, and emistim C «switches on» genes under strong force promoters in the morning with expression up to the level shown under bright (strong) sunlight.

Key words: plant growth regulator emistim C, mRNA populations, photosynthetical activity.

So far it is known that the majority of genes in the plant cells (as and in the cells of animals) are represented by families. For example, multifamily of photomorphogenesis genes [1–5]; phospholipase C gene — out of 9 members [6–8]; phospholipase D — out of 12 members [9]; patatin genes — out of 70 members on tetraploidy genome *Solanum tuberosum* L. [10–14]; genes of extensin cell wall protein – out of 5 members [15–17]; RNases genes are represented by super- or multifamily [18] etc.

Each member of a gene family differs from each other by microheterogeneity in a nucleotide sequence at variable and encoding parts of genes and in mechanisms of their intracellular regulation accordingly [6–9]. Mini differences in the structures of gene products — proteins (polymorphism of reserve proteins, isoenzymes etc.) are reflection of structural microheterogeneity in the gene families as well [19, 20].

We supposed that variability in the structures of the gene family members, difference in regulation mechanism of their activity and mini differences in the structures of the end-products of gene expression respectively provide high mobility and plasticity of adaptive reactions of the cells to the environment signals.

Materials and methods

The leaves of dicotyledonous (soya, pea) and monocotyledonous plants (wheat, corn) were used for ten-day period of their vegetation followed by initiation of seed germination growing in environmental (field) standard conditions and first of all at strong clear and weak sunlight as a subject of inquiry. The experiments were conducted at the beginning of June when the most optimal growth and development of plants occur. The preparations of cytoplasmic mRNA were selected from the plant leaves in different periods of daylight hours: in the morning (at 5 a. m.) — in the period of dawning light; then in the noonday (at 2 p. m.) — during maximal sunlight and in the evening (at 9 p. m.). The preparations of cytoplasmic mRNA out of leaves were as a control during lack of lighting in the middle of nighttime (at 2 a. m.).

Experiments were repeated every three consecutive days in order not to impose divergence indexes of mRNA homology due to the stage changes of mRNA populations. The degree of mRNA homology out of leaves was determined on the farther plant development stage (over subsequent 10 days) with relation to the same mRNA control preparation in order to determine the stage distinctions.

Polycomponent preparation emistim C, which is alcoholic extract of mushrooms-micromycetes containing physiologically active substances (auxins, gibberellins, cytokinins, amino acids and microelements), was used as a growth regulator. Different countries of the world such as Germany [21], China [22, 23] and others use widely this preparation in their agricultural practice.

Isolation of total preparation of cytoplasmic RNA. The plant tissue was homogenized in a buffer solution I (containing 0.05 M Tris-HCl, pH 7.6; 0.01 M $MgCl_2$; 0.06 M KCl; 1% SDS and 4M guanidine isothiocyanate). The obtained lysate was centrifuged under 1 500 g and twice treated by a mixture of hot (80 °C) water-saturated solution of phenol with chloroform; the RNA preparation was precipitated from a water phase by ethanol [24, 25].

The RNA precipitate was dissolved in 0.025 M Tris-HCl buffer, pH 8.1, containing 0.025 M NaCl [24, 25]. The solution was cooled and one of its volume was added to the equal volume of 2.5 M potassium-phosphate buffer, pH 8.0 and to one volume of 2-methoxyethanol for the purpose of polysaccharide precipitation. The mixture was shaked up vigorously under 2 °C and centrifuged during 5 min at 10 000–12 000 g. The diaphanous upper layer was cautiously selected and mixed with the equal volume of 0.2 M sodium acetate, after this procedure RNA was precipitated under 2 °C with 1% solution of cethyltrimethylammonium bromide (CTAB) in amount of 0.5 ml per 1 ml of upper phase after centrifugation.

The suspension was incubated under 2 °C during 5 min and the precipitate was separated by centrifugation during 5 min under 12 000 g. Supernatant was drained and the precipitate of CTAB-nucleate was flushed twice with 70% ethanol excess contained 0.1 M potassium-acetate in order to remove CTAB and to put RNA into potassium salt. After ethanol flushing the pellet was stored under low temperature (–70 °C).

Purification degree of isolated RNA preparations was determined with spectrophotometer via measuring E_{260}/E_{280} and E_{260}/E_{230} ratio that was ≥1.9 and ≥2.3 respectively. This fact indicated high degree of purification of isolated RNA preparations that contained no proteins and polysaccharides [24].

Separation of Poly(A)$^+$ mRNA from Poly(A)$^-$ mRNA molecules was carried out using total RNA chromatography on the oligo(dT)-cellulose columns [26, 27]. Glass columns of 5 ml volume having glass-filters packed in the lower part were filled with oligo(dT)-cellulose mash suspended in a buffer B (containing 20 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 1 мМ of EDTA). All of these were mixed, autoclaved and later on the SDS was added into these columns to 0.1%. 3 ml of 0.1 M NaOH with 5 mM EDTA were flown trough the columns, then the columns were washed by H_2O for pH diminishing below 8. Afterwards the column was counterbalanced with 5 ml buffer A (containing 40 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 1 мМ of EDTA), autoclaved and then 0.1% SDS solution was added.

For duplexes destruction the RNA preparation (10 mg) was dissolved in 0.14 M NaCl, heated up to 65 °C during 5 min, then buffer A (500 µl) heated to 65 °C was added, mixed and cooled to the room temperature during 2 min 1 ml of this probe was brought into a column, the eluate was collected, heated up again to 65 °C during 5 min and cooled to the room temperature during 2 min and put once again into a column followed by washing with 5 ml of buffer B. This eluate contained no poly(A)$^+$ RNA was wasted. Poly(A)$^+$ RNA was removed from a column with 2–3 volumes of buffer containing 10 mM Tris-NaCl (pH 7.5), 1 mM EDTA, 0.05% SDS solution [27, 28].

Polymerity of isolated RNA was analysed by electrophoresis in 1.5% agarose gel containing 7 M urea according to Locker [29]; the obtained gels were saturated by ethidium bromide solution before RNA fractions photographing using an UV lamp.

Singl-stranded P^{32} cDNA synthesis. The mRNA of control leafs was used for cDNA synthesis. The cDNA was synthesized on poly(A)$^+$ mRNA templates [27, 30] using reverse transcriptase and [α-^{32}P]-labelled dCTP. For cDNA-strand synthesis it was used buffer solution containing: 100 mM Tris-HCl (pH 8.3), 10 mM $MgCl_2$, 140 mM KC1, 100 mg/ml oligo(dT)$_{12-18}$ primer, 2 mM methylmercuric hydroxide, 20 mM β-mercaptoethanol, 1 mM solutions of all four $dNTP_s$, 100 mg/ml poly(A)$^+$RNA, 800 units/ml reverse transcriptase and [α-^{32}P]-dCTP (800 Ci/mM), 1 M Tris-HCl (pH 8.3) at 42 °C.

The poly(A)$^+$RNA was analysed using a Northern-blot approach [27, 28]. A poly(A)$^+$RNA preparation was fractionated by electrophoresis in an agarose gel in the presence of formalin (5% solution of formaldehyde); elecrtophoretically pure poly(A)$^+$RNA fractions were transferred on nitrocellulose filters and hybridized with cDNA. cDNA was synthesized on poly(A)$^+$RNA template according to [27, 28] using reverse transcriptase (revertase) and [α-^{32}P]-containing deoxy-CTP as a label. For cDNA synthesis the buffer solutions

containing 100 mM Tris-HCI (pH 8.3) at 42 °C, 10 mM $MgCl_2$, 140 mM KC1,100 μg/ml oligo$(dT)_{12-18}$, 2 mM methylmercuric hydroxide, 20 mM β-mercaptoethanol, 1 mM vanadyl-ribonucleoside complexes, or 0.5 units/μl RNasin, 1 mM of each dNTPs, 100 μg/ml poly$(A)^+$ RNA, 400–800 units/ml of reverse transcriptase and [α-^{32}P]-dCTP (800 Ci/mM) were used. The hybridization mixture contained 50% formamide, 5X Upper buffer (pH 7.0), 5X Denhardt's solution (5g ficoll, 5g polyvinyl-pyrrolidone, 5 g BSA (Penta, Fraction V), 500 ml H_2O), denaturated calf thymus DNA (100 mg/ml), poly$(A)^+$RNA (1 mg/ml), 0.1% SDS, and [α-^{32}P]-cDNA ($1{,}5\cdot10^8$ cpm) [27, 31, 32]. After hybridization (throughout the day), the filters were carefully washed free from exogenous label and exposed during 24 h to a film PM-1 with an intencifying screen (at −70 °C). The limits of poly$(A)^+$RNA sizes were evaluated according to radioautograph distribution of labeled poly$(A)^+$RNA-cDNA hybrids along the gel lanes (with regard to marker polynucleotides and fractions of total RNA).

DOT-blot hybridization P^{32} cDNA with mRNA. The mRNA preparations out of experimental plants were subjected to hybridization with cDNA using DOT-blot method [27, 28] for the purpose of the percentage test for mRNA population's homology out of experimental plants in regard to mRNA out of control plants. According to a scheme given above the experiments were repeated daily during 3 days in order the indexes of mRNA homology divergence were not overlapped due to the stage change of mRNA populations. The mRNA homology degree out of leaves at the farther stage of plant development (in 10 days) relative to the same control mRNA preparation for the phase difference definition was investigated as well [33].

For DNA deposition on the filters the first one was dissolved up to a concentration 5–10 μg/ml in 0.3 M NaCl — 0.03 M Na citrate solution, pH 7.0 (2XSSC). For cDNA hybridization with mRNA, cDNA in quantity 20 μg for each probe was deposited on modified and activated cellulose filters (Whatman 50, 2-aminophenylthioether paper) that forms covalent linkages with deposited DNA or RNA unlike the cellulose and nitrocellulose filters that form hydrogenous bonds with DNA or RNA enabling to avoid losses of the nucleic acids in the course of the filters washing out [24, 27].

For hybridization on the filters, cDNA with mRNA were placed into the cuvettes, that were used for determination of radioactivity in LS 100C scintillation counter «Beckman», filled with 2XSSC solution with tenfold excess of mRNA as to cDNA [27, 28]. In each experiment the filters containing DNA out of genetically distant source for control on hybridization specificity and the filters, contained no DNA were placed in separate cuvettes with a view to estimate value of non-specific RNA sorption on filters material. The vials with filters were tightly closed and put into a thermostat for hybridization.

Hybridization was carried out at 66 °C, however during work with nucleic acids out of different sources we took into account that RNA molecules being of more resistant second structure (due to lot GC-pairs in its content that possibly takes place in total mRNA preparation) require higher temperatures. An optimum temperature and maximal level of hybridization were selected by means of mRNA hybridization level measuring according to homologous and heterologous cDNA. Hybridization was compared in different time intervals within 24 hours.

After termination of hybridization the vials with filters were cooled, the filters were taken out from the dishes filled with hybridization solution and washed in a watering can from every side with 50 ml 2XSSC solution. After washing the filters were placed in 2XSSC solution containing RNase at a final concentration 20 μg/ml. After incubation with RNase at a room temperature during an hour the filters were washed out again with 2XSSC solution, then with ethanol. Radioactivity of the probes was determined using Millipore AP-15 fibrous glass filters at toluene scintillator in a LS 100C scintillation counter «Beckman».

Results and discussion

Land plants are anchored in one place for most of their life cycle and therefore must adapt their physiology and development to abiotic stresses such as light intensity, drought, salinity, availability of nutrients, extreme temperatures and physiologically active compounds, which regulate the expression of thousands of genes in plants at both transcriptional and posttranscriptional levels [34, 35].

We point out at studying degree of homology of «mature» cytoplasmic mRNA populations in plant leaves at different intensity of sunlight.

It is known that a transcription initiating site is a regulatory sequence of gene promoters on which the forming of initiating transcription complex (transcriptosome by analogy

with a ribosome) takes place including the regulator elements of promoter RNA-polymerase II, and a great number of *trans*-acting factors of protein nature (about 70) [36] one of which serves for perception and realization of external signals in the processes of initiation transcription, other are for correction of these processes on the part of other regulator areas of genes — silencer and enhancer sequences of DNA providing necessary speed and level of transcription (copying predecessors of mRNA with DNA template — coding part of genes).

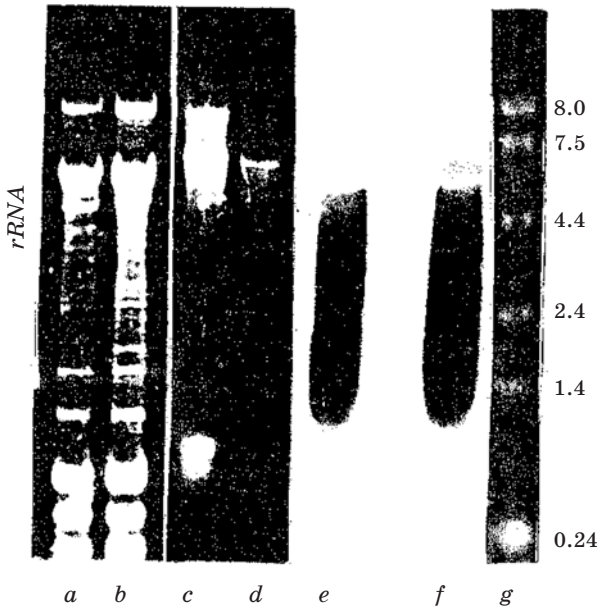
It is important to note that one of the fundamental elements of initiating transcription complex is a structure (nucleotide sequence) of regulation areas, foremost promoter, which determines a level and duration of gene transcription cycles. In this connection all the promoters of structural genes (organo-specific including) are divided on their activity into strong promoters, moderate force and weak promoters. Strong promoters are constitutive in majority (workings as promoters of viral and bacterial genes continuously, for example, gene of metallothionein) [37–39].

So far about 50 promoters of plant genes are cloned and sequenced in the world [36]. Two organo-specific promoters — a root-specific promoter of moderate force and a strong promoter of patatin gene of the I-st class with enhancer were cloned by employees of our laboratory jointly with the employees of the Institute of Molecular Genetics of Russian Academy of Sciences [10, 40].

We supposed that variants of genes from gene families forming an answer for weak external signals (for example, to a weak thermal, light or chemical signals) are under weak promoters; genes forming an answer for the signals of middle force are located under promoters of moderate force, and genes from the gene families forming the return reactions of cells on strong external or internal signals (for example on maximal photosynthesis in the period of strong sunlight and strong heating accordingly) are under strong promoters.

According to this supposition, increase of photosynthetic process occurs as a result of «windmill switching» of the corresponding genes under weak promoters on genes with promoters of moderate force and further on genes under strong promoters in process of increase in solar activity and vice versa the opposite process «switching» genes is observed, when solar activity decreases.

With a view to avoid the artifacts connected with the possibility to obtain degradated mRNA preparations at estimating degree of



Agarose gel electrophoresis of RNA preparations isolated from embryo cells of germinating pea seeds:

a and *b* — total RNA preparations from control and emistim C-stimulated (experiment) pea seeds, respectively; *c* and *d* — electrophoresis of poly(A)$^-$RNA and poly(A)$^+$RNA preparations out of control embryo cells, respectively, separated on oligo(dT)-cellulose column; *e* and *f* — radioautographs of hybrid molecules containing [^{32}P]-cDNA and poly(A)$^+$RNA fractions immobilized on nitrocellulose filter isolated out of embryo cells of non-stimulated (*e*) and emistim C-stimulated (*f*) germinating pea seeds, respectively; *g* — electrophoretic distribution of marker polynucleotides on a parallel gel lane

Table 1. **Percent of cytoplasmic mRNA populations homology out of plant leaves during different periods phase of photophase (morning, day and evening) (experiment) concerning mRNA of dark (midnight) phases (control)***

Time	Test objects and variants of the experiments											
	Soya bean			Pea			Wheat			Corn		
	light	light + emistim C	over 10 days	light	light + emistim C	over 10 days	light	light + emistim C	over 10 days	light	light + emistim C	over 10 days
5:00AM	95±2.2	84±1.9	86±1.7	97±2.1	84±1.6	89±1.4	91±1.6	82±2.2	88±1.3	94±1.5	86±2.1	85±2.1
2:00 PM	86±2.3	78±1.8	81±1.8	85±1.4	79±1.9	84±1.3	79±2.3	75±1.8	86±1.6	79±2.1	76±1.8	78±1.7
9:00 PM	89±1.4	84±1.6	78±1.3	94±1.8	83±1.6	86±2.1	93±1.4	86±1.3	88±1.8	92±1.9	84±1.7	87±1.4

*Percent of cytoplasmic mRNAs homology in relation to control mRNA preparation (taken for 100%) was determined by index differences on hybridization level of P^{32} cDNA with mRNA populations, which are changed during a day and differ by nucleotide sequence in variable part of gene structure within the family limits. Hybridization level of P^{32} cDNA (copies from «nightly» mRNA) is accepted for 100% (control) with «nightly» mRNA of plant leaves (hybridization «on itself»). Experimental error is ± 2% (the average data from 3 experiments are given). Unit of measurement is a level of radioactivity in impulses at each probe.

mRNA homology between control and experiment, we conducted the experiments on verification of structural entirety of isolated mRNA preparations (studying conformity of molecular masses mRNA through cDNA according to the indexes cited in literature for total eukaryotic mRNA preparations). On the figure there are data obtained by Northern-blot method [27, 28] concerning the limits of distribution in agarose gel of all gamma mRNA molecules hybridized with cDNA.

Thus it was shown that according to spectrum sizes of molecular masses the isolated mRNA preparations are present at limits of mRNA sizes of different eukaryotic organisms, i. e. they are nondegradated mRNA molecules.

In the table 1 there are results witnessing that both dicotyledonous and monocotyledonous plants show a small decline of homology degree of mRNA in an experience as compared to control (up to 91 percent for different plants) with the advent of sunrays in the early morning (at 5 a. m.).

In the middle of day (at 2 p. m.) there is yet greater divergence between control («nightly») mRNA and mRNA from the plant leaves at more intense lighting.

Towards evening it seems likely that photosynthetic processes reduction takes place via «windmill switching» of the genes under strong promoters on genes under weak promoters. The difference in mRNA populations from leaves of control plants and plants on the 20-th day of vegetation after beginning of the experiments is observed. It could be interpreted possibly by «switching on» in expression partially other genes providing ongoing processes of differentiation of plant cells on subsequent stages of their vegetation [33].

It was shown in E. N. Tischenko and O. V. Dubrovnoy works that when transition of sugar beet seedlings from the etiolated state into photomorphogenesis took place, approximately 2 000 specific poly(A)$^+$mRNAs appear in the cotyledons of seedlings and about 400 specific poly(A)$^+$mRNAs disappear, i.e. there is a sharp reprogramming of plant cells genome during their transition from etiolated stage into photomorphogenesis one. The authors received similar results on sunflower as well [41, 42].

H. Price and J. Johnston revealed reduction of DNA content in the cells of *Helianthus annuus* Linnaeus plant under the influence of light [43].

Together with these experiments we also studied the emistim C growth regulator influence on population descriptions of cytoplasmic mRNA in periods of days referred to above aiming to check whether premature genes «switching on» takes place under strong promoters during weak lighting instead genes «switching on» under weak promoters or simultaneously of both. It is based on the experiments with coffee plants sprouting in laboratory apartments with room lighting and producing coffee grains. Watering of these plants with emistim C solution caused sharp increase of plant productivity (3 crops of cof-

Table 2. **Influence of growth regulator of emistim C on structure-morphologic characteristics and productivity indexes of winter wheat** (2009 year)

Indexes	Control	Emistim C	Increase (%)
Total leaf area of 1plant (cm^2)	90.8 ± 1.6	108.3 ± 1.8	19.3
Total plant leaf area from 1 hectare (m^2)	4 358.0 ± 1.2	53 500.2 ± 1.4	22.8
Chlorophyll content in mg in 1 g moist plant (mg/g)	3.0 ± 0.1	4,1 ± 0.2	36.7
Grain yield in centner from 1 hectare (c/ha)	43.1 ± 0.1	55.4 ± 0.2	28.5

fee grains were got for a year instead of one) forming rifle-green leaves at law room lighting that was usually observed at strong lighting. *In vitro* experiments it was shown as well that cultivation of tobacco calluses in darkness on a medium containing either ivin-yan or emistim C caused their greening and delayed their aging process (approximately in 2 times) [44].

As part of study, it was found that the plants treated by emistim C showed considerably less decreased photosynthetic activity. Distinctions in mRNA populations between the control plants and that on the later stages of ontogenesis in mRNA populations were related to inclusion in work partly already other genes providing the forward processes of plant cell differentiation.

Preprocessing of plants by emistim C intensifies significantly difference in the degree of mRNA homology of control and experimental plants already at 5 a. m. Increasing of photosynthesis processes by emistim C even at a weak sunlight is indicative of it.

Numerous obtained results concerning considerable increasing almost on 36% of chlorophyll in the plant leaves under the influence of emistim C (table 2), increasing of thickness of leaves epidermis, size of lamina and strengthening of structure of plant conducting vascular bundles [45] say for it.

However, it was observed no sharp distinctions in mRNA population out of daily plants and that of the treated with emistim C and also 10 days later after further vegetation (i. e. 20 days later after beginning of the experiment). It goes to prove that expression of genes under strong promoters is maximum at noontime but whether the same or different gene type are actively expressed in leaf cells of noon plants as well as of plants of more extended vegetation period one could not say jet on the basis of the data obtained.

Numerous works are devoted to the investigation of molecular mechanisms of regulation of gene expression under light [1, 3, 5]. It is found that photosynthesizing organisms use complex biochemical systems perceiving and responding to different light waves. Different classes of photoreceptors have been identified in higher plants: the red/far-red light-absorbing phytochromes and red light-perceiving chlorophyll; the blue/UV-A/UV-B selectively light-absorbing photoreceptors: cryptochromes, phototropins and carotenoids. As it was shown in the reviews [3, 5], the light signals were absorbed by these photoreceptors and transduced by associated molecular systems regulating the expression of many genes both at the transcriptional and posttranscriptional levels. These include genes encoding photoreceptors phytochromes (PHYA — PHYE genes), cryptochromes (CRY1 and CRY2 genes) and phototropins (NPH and NPL1 genes) in *Arabidopsis*. In these works the emphasis was upon consideration of concrete genes which expression was regulated by light signals through phytochrome A (FHY1, FHY3, SPA1, FIN2, FIN219, FAR1 genes); phytochrome B (RED1, PEF2, PEF3, PKS1, ATHB-2 genes) as well as through both phytochrome A and phytochrome B (PEF1, PSI2, PIF3, NDPK2, HY5, Elip genes). The data concerning discovery of the following new genes: COP1, DET1, COP9, COP10 and SHL — negative regulators of morphogenesis in the dark, which expression is regulated by two classes of photoreceptors such as phytochromes and cryptochromes; the information concerning identification of numerous genes transmitting the environmental signals to molecular physiologic clock (endogenous oscillator): ZTL, FKF1, LKP2, TOC1, CCA1, LHY, ELF3, GL, PHYA — PHYE, CRY1 and CRY2 genes transmitting the environmental signals onto an oscillator: CAB2, CCR2, CAT3, psbA, psbD genes etc. were described as well; the evidences about signal interaction of phytochromes and phytohormones were given [3, 5].

Numerous *cis*-regulatory promotor elements and *trans*-acting factors take part in regulation of plant gene light-induced transcription. As it was shown in the work [1], the 30 various classes of structurally and evolutionally conserved DNA module sequences (CMAs) associated with light-responsive elements (LREs) of promoter regions were identified by a new phylogenetic-structural method and it was analyzed more than 110 light-regulated plant genes. It is found out that the identified CMAs share a gene superfamily-specific core that correlates with the phytochrome-dependent transduction pathway controlling their expression, i.e. ACCTA(A/C)C(A/C) sequence for cGMP-dependent phenylpropanoid metabolism-associated genes (superfamily PhMAGs genes encoding anthocyanin biosynthetic enzymes, i. e. *chs*), and GATA(A/T)GR for Ca^{2+}/calmodulin-dependent photosynthesis-associated nuclear genes (PhANGs genes superfamily coding chloroplast protein, i. e. *rbcS*). According to hypothetical model of structural and functional organization of PhANGs and PheMAGs LREs [1], light is absorbed by phytochrome photoreceptors, and subsequently the components of the associated transmission system influence successively on specific transcriptional factors which become either active or inactive according to their regulatory function. In compliance with the positive regulation scenario, both the IB/LAMP-binding factors (IBFs), that are compatible with IB/LAMP-related modules (light-specific elements LREs of PhANGs genes) and H-box binding factors (HBFs) which interact with H-box-related motifs (light-specific elements LREs of PheMAGs genes), are light-activated and are able to attach either independently or associated together with other transcriptional factors (forming heteromeric regulatory complex) to corresponding DNA cognate sites (LiS elements). When proper exogenous and/or endogenous signals are concurrent with the light stimulus, the photoresponsive unit can activate gene transcription. Subject to a negative regulation scenario, IBFs and nuclear-bounded factors interact functionally and activate transcription independently of photoimpact. However in light absence the regulators like those of de-etiolated/constitutive photomorphogenic plants interact with IBFs forming a multicomponent repressor complex.

The data of our researches enable to suppose that emistim C «switches on» those genes of photomorphogenesis that are expressed in its active phase (in the noontime) and under normal conditions they start to work under specific spectrum of sunlight, which is inherent to this time-of-day. Thus obviously presence of the gene families and specific mechanisms of their regulation provide «windmill switching» genes (under influence of one or another signals) executing the same functions but possessing microheterogeneity on nucleotide sequences and different levels of activity that permits to consider them as natural mutants relative to each other). It provides plant adaptation to the varied environment and reproduction of individuals to similar itself in a greater or less amount under different conditions for maintenance and saving in nature of one or another specific plant population. The action result of emistim C growth regulator is achieved (see table 1) by the way of premature «switching on» genes (unforeseen by nature in this period) with high activity under strong promoters in the conditions that are inadequate for their function resulting in acceleration of plant growth and development. As a result their productivity is increased even in unfavorable conditions commensurable with such data that could be obtained without using growth regulators in optimal light conditions.

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ПРО ОСОБЛИВОСТІ ЗМІН ЕКСПРЕСІЇ ГЕНІВ У КЛІТИНАХ ЛИСТКІВ РОСЛИН УПРОДОВЖ ДОБИ

В. А. Циганкова

Інститут біоорганічної хімії та нафтохімії НАН України, Київ

Методом ДОТ-блот-гібридизації ДНК із РНК проведено аналіз ступеня гомології продуктів експресії генів (цитоплазматичних мРНК) з клітин листків дводольних (сої, гороху) й однодольних (пшениці, кукурудзи) рослин після відділення їх від рослин в ранній період (о 5-й год ранку) зі слабким фотосинтезом, в середній період (о 14-й год дня) із сильним фотосинтезом і в вечірній період (о 21-й год вечора) зі згасаючим фотосинтезом стосовно мРНК листків рослин, що перебували в темновій фазі (о 2-й год ночі — контроль). Показано, що різниця в популяціях мРНК між дослідом і контролем починається вже вранці за слабкого сонячного освітлення, різко посилюється при денному освітленні (о 14-й год дня) та знижується на заході сонця. Емістим С посилює розходження між контролем та 5-годинним періодом доби до рівня 14-годинного періоду. Припускають, що ці зміни відбуваються шляхом «віяльного» переключення генів, що належать до однієї родини, але виявляють різну активність через розташування їх під промоторами різної сили (слабкої, помірної та сильної), і що шляхом такого механізму досягається пластичність (маневреність) адаптації рослин до навколишнього середовища, а емістим С вмикає гени під сильними промоторами в ранній період доби з експресією до того рівня, який спостерігається за сильного сонячного освітлення.

Ключові слова: регулятор росту рослин емістим С, популяції мРНК, фотосинтетична активність.

ОБ ОСОБЕННОСТЯХ ИЗМЕНЕНИЙ ЭКСПРЕССИИ ГЕНОВ В КЛЕТКАХ ЛИСТЬЕВ РАСТЕНИЙ НА ПРОТЯЖЕНИИ СУТОК

В. А. Цыганкова

Институт биоорганической химии и нефтехимии, НАН Украины, Киев

Методом ДОТ-блот-гибридизации ДНК с РНК проведен анализ степени гомологии продуктов экспрессии генов (цитоплазматических мРНК) из клеток листьев двудольных (сои, гороха) и однодольных (пшеницы, кукурузы) растений после отделения их от растений в ранний период (в 5 ч утра) со слабым фотосинтезом, в средний период (14 ч дня) с сильным фотосинтезом и в вечерний период (в 21 ч вечера) с угасающим фотосинтезом по отношению к мРНК листьев растений, находящихся в темновой фазе (2 ч ночи — контроль). Показано, что разница в популяциях мРНК между опытом и контролем начинается ранним утром при слабом солнечном освещении, резко усиливается при дневном освещении (14 ч дня) и угасает на заходе солнца. Эмистим С усиливает расхождение между контролем и 5-часовым периодом суток до уровня 14-часового периода. Предполагается, что эти изменения происходят путем «веерного» переключения генов, принадлежащих к одному семейству, но обладающих разной активностью из-за расположения их под промоторами разной силы (слабой, умеренной и сильной), и что путем такого механизма достигается пластичность (маневренность) адаптации растений к окружающей среде, а эмистим С включает гены под сильными промоторами в ранний период суток с экспрессией до того уровня, который наблюдается при сильном солнечном освещении.

Ключевые слова: регулятор роста растений эмистим С, популяции мРНК, фотосинтетическая активность.