Plant Physiology and Biochemistry 61 (2012) 9–17

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Levels of DNA methylation and histone methylation and acetylation change in root tip cells of soybean seedlings grown at different temperatures

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ARTICLE INFO

Article history:
Received 29 May 2012
Accepted 1 September 2012
Available online 23 September 2012

Keywords:
Chilling stress
Chromocentres
DNA methylation
Histone modifications
Soybean root tip nuclei

ABSTRACT

In order to check whether changes in DNA and histone modifications occur in the nuclei of root tip cells of soybean seedlings grown 1) under control conditions (25 °C), 2) subjected to chilling stress (10 °C) and 3) recovered (25 °C) after chilling, measurements of fluorescence intensity with the use of antibodies to heterochromatin as well as to euchromatin markers were carried out. Moreover, the number and sizes of chromocentres were analyzed. The studies showed that during chilling stress the fluorescence intensity for the markers characteristic of heterochromatin increased while for the markers of euchromatin decreased in comparison to the control. After the recovery the converse situation was observed, i.e. increase in fluorescence intensity for euchromatin markers and decrease in heterochromatin markers. The number of chromocentres remained unchanged in the nuclei of all three studied variants. However, differences in the sizes of chromocentres were observed – the highest number of big chromocentres and simultaneously the lowest number of small chromocentres were in the nuclei of stressed plants. Conversely – in the nuclei of recovered plants there were the lowest number of big chromocentres and the highest number of small ones. The treatment of seedlings with the inhibitors of DNA methylation (5-aza-dC) and histone deacetylation (NaBu) also caused changes in fluorescence intensity and chromocentre sizes in soybean nuclei. These results suggest that DNA and histone modification patterns can be altered in soybean nuclei by different growth temperatures and by appropriate inhibitors influencing epigenetic chromatic modifications.

1. Introduction

Eukaryotic DNA is plaited into chromatin, which generally consists of condensed heterochromatin and loose euchromatin. Euchromatin regions are usually rich in genes and competent for transcription, while heterochromatin regions are rather gene-devoid and transcriptionally incompetent. The relation between potentially active and inactive genes is governed by the epigenetic mechanism controlling gene expression and is related to chromatin organization. Combination of histone modifications and the information encoded by DNA methylation determines the structure of chromatin and plays a crucial role in the regulation of gene expression [1,2]. Changes in covalent modifications of histones and the level of DNA methylation can alter the architecture of chromatin and change the accessibility of DNA to the transcriptional machinery [3–5] which means that chromatin reorganization could lead to switching some genes on or off. Chemical modifications of chromatin components which can alter its organization include 1) posttranslational histone modifications – acetylation, methylation, phosphorylation and ubiquitination which constitute a histone code [6] and 2) changes of the level of DNA methylation at CpG sites [7] and in plant-specific CpNpG and CpNpN sites (N = A, C or T) [8]. Cytosine DNA methylation is a prominent epigenetic marker in most eukaryotic organisms which plays an important role in maintaining genome stability and controlling gene expression especially as a global repressor of gene expression [9]. The acetylation of lysine residues on histone tails is also strongly correlated with transcriptional activation [10]. Hypermethylation of cytosine residues in DNA and of histones H3K9 and H4K20 as well as hypoacetylation of histone H4 are usually correlated with silenced chromatin, whereas hypomethylation of DNA and high level of triple methylated histone H3K4 (H3K4met3) and heavy acetylated histone H4 are thought to correlate with transcriptionally active rDNA chromatin [11–13]. Obviously, different species may display deviations from generally accepted rules and differences concern mainly the number of methylated or acetylated groups and position of modified amino acids in histones [14]. Regulation of transcriptional gene activity

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http://dx.doi.org/10.1016/j.plaphy.2012.09.001

through chromatin organization involves not only DNA (de)methylation, (de)acetylation or (de)methylation at different H3 and H4 lysine residues but it is also complemented by recruitment of special proteins such as heterochromatin protein 1 (HP1) [15] in animals or its homolog, like heterochromatin protein 1 (LHP1) in *Arabidopsis* [16]. Activity of some interfering RNAs is an additional mechanism participating in the epigenetic regulation of gene expression also in response to external stimuli [17].

Chromatin modulations and subsequent up or down regulations of many genes occur during normal physiological and developmental processes in most eukaryotes. In plants chromatin regulators control the transitions between successive developmental stages and include such processes as: seed size regulation [18], development [19] and germination [20], cell differentiation [21], root elongation [22], potato tuber dormancy [23]. Many developmental processes during which chromatin remodeling occurs are connected with perception of inductive environmental signals. Recent data show that changes in DNA methylation in plants, leading to chromatin remodeling, also play an important role in gene expression in response to external environmental stimuli not associated with physiological development but as a cellular response to biotic and abiotic stresses [24–26]. Pathogen attack changes the level of histone methylation and acetylation in plants and these modifications could provide epigenetic memory for SAR (systemic acquired resistance) [27]. Under spaceflight conditions the hypermethylation of DNA at CG and CNG sites as well as changes in gene expression occurred in rice plants [28]. Chronic irradiation of wheat seeds resulted in increase in DNA methylation level [29]. Transition from dry to wet seasons induced seasonal changes in H3K4met2 and H3K9met levels in a desert plant *Zygophyllum dumosum* which was associated with drought tolerance [30]. Chromatin modifications associated with chromatin remodeling were observed in plants in response to cold. Increases in histone H3 deacetylation and in methylation of H3K9 and H3K27 were shown to control cold-induced plant flowering response, a process known as vernalization achieved by prolonged exposure to cold [31] as well as to regulate plant cold tolerance and acclimation [32]. During cold, salt and abscisic acid treatments the nucleosomal response including changes in core histone H3 and H4 phosphorylation and acetylation was observed in tobacco and *A. thaliana* cells [33]. In *Z. mays* root tissues the level of DNA methylation changed in the cores of nucleosomes in response to cold stress and this might modify gene expression [34].

Here soybean root meristem interphase nuclei were investigated with regard to chromatin organization, hence it is worth mentioning that this plant has been supposed to undergo two rounds of large-scale genome and/or segmental duplication (allopolyploidization) [35]. Soybean root meristematic nuclei belong to euchromocentric nucleus type with small DNA content of 2C DNA = 2.43 pg [36] and 2n = 40 small-sized chromosomes [37]. The size of sequenced soybean genome is 1.1 Gb/1C DNA. 57% of the total genomic sequences occur in repeat-rich heterochromatic regions, while the rest of the genome consists of euchromatic, gene-rich sequences [38].

Moreover, soybean is sensitive to low temperature. Earlier studies showed that soybean root tip cells strongly responded to temperature of 10 °C which was manifested in alterations of nucleolar structure and functions [39,40]. It was also observed that under such conditions a drastic decrease in transcriptional activity of soybean cells occurred, judging from the reduced incorporation of a radioactive precursor of RNA synthesis, ^{3}H-uridine, in comparison to the optimal conditions (the control, 25 °C). On the other hand, in the stressed plants recovered at the optimal temperature (25 °C) increase in the radioactive precursor incorporation, even in comparison with the control, occurred [41,42]. The assumption has been made that changes in transcriptional activity could result from alterations of chromatin organization at these temperatures. Hence, the aim of the current work was to check whether the chemical modifications of chromatin and its structural organization change in nuclei of soybean root meristem cells along with the change of temperature during plant growth. Thus the immunofluorescent studies with the use of antibodies against methylated DNA (5-methylcytidine) and methylated histone (H3K9met2) – repressive chromatin marks, as well as acetylated (H3K9acetyl, H4K12acetyl) and methylated (H3K4met3) histones, which are indicators of permissive chromatin were carried out.

2. Results

2.1. Chilling stress- and post chilling recovery-induced changes of fluorescence intensity and staining pattern of nuclei

2.1.1. 5-Methylcytidine

Nuclei contained several (or a dozen or so) clear immunosignals in a form of small separate spots of intensive labeling (corresponding to chromocentres) against a background of weaker labeled nuclear territory. These foci overlapped with intensively DAPI stained nuclear regions (Fig. 1A, A′). Such images implicate a high level of DNA methylation in heterochromatic regions. The highest fluorescence intensity was observed in the nuclei of chilled plants (about 26% more than in the control), the weakest – in the nuclei of recovered seedlings (about 18% less in comparison to the control). This means that the level of DNA methylation increased in the nuclei of stressed plants, which may suggest augmentation of condensed chromatin amount. Converse situation was observed during recovery, decrease in DNA methylation and possibly in the amount of heterochromatin. The use of DNA methylation inhibitor weakened fluorescence intensity in all experimental variants by about 25% (Fig. 1A′; Table 1).

2.1.2. H3K9met2

The whole territory of extranucleolar nucleoplasm was covered with immunosignals but with different intensity. There were spots more intensively labeled which overlapped with the nuclear areas more intensively stained with DAPI (Fig. 1B). Such a pattern of labeling indicated that histone H3K9met2 was present almost in all chromatin but its highest level occurred in heterochromatin. The highest intensity of immunofluorescence was observed in the nuclei of plants under chilling stress (about 34% more than in the control), while the weakest – in those of recovered plants (about 17% lower than in the control) (Table 1). This result confirms the fact that the amount of condensed chromatin increased in the nuclei of plants subjected to chilling, and decreased when plants were recovered.

2.1.3. H3K4met3

The nuclei were heterogeneously stained – there were areas of intensive and weak labeling as well as areas free of staining. The latter overlapped with nuclear areas of intensive DAPI staining (Fig. 1C). Such labeling could indicate that the antibody to H3K4met3 disclosed mainly euchromatic regions in the nuclei of soybean root tip cells. The nuclei of recovered plants were most intensively labeled (about 18% more than in the control), while those of the plants subjected to chilling stress showed the weakest labeling (about 24% less than in the control) (Table 1). This could mean that the quantity of euchromatin increased in the plants recovered at optimal temperature, while it decreased in the nuclei of stressed plants.