Expression of Glutamate Dehydrogenase Genes In *Arabidopsis Thaliana* Depends on The Redox State of Chloroplast Electron Transport Chain

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Abstract

Plant glutamate dehydrogenase is an enzyme interconverting L-glutamate and 2-oxoglutarate and providing a link between carbon and nitrogen metabolism. In *Arabidopsis thaliana*, this enzyme is encoded by three genes. Two of them, *GDH1* and *GDH2*, provide most of the enzyme activity in plant leaves and roots. Expression of *GDH1* and *GDH2* genes is very low in the light and high in the dark. The molecular signals and mechanisms that provide the light-dependent *GDH* genes regulation remain unknown. Using photosynthetic electron transport inhibitors 3-(3.4-dichlorophenyl)-1.1-dimethylurea (DCMU) and 2.5-dibromo-3-methyl-6-isopropyl benzoquinone (DBMIB) we demonstrate that transcript levels of the *GDH1* and *GDH2* genes in *Arabidopsis* leaves change in accordance with a redox state of chloroplast electron transport chain: they are low when it is highly reduced and high when it is oxidized. Hydrogen peroxide or high light treatment did not result in decreasing of *GDH1* or *GDH2* expression, so reactive oxygen species cannot be the signals that reduce expression of these genes during dark-to-light shifts. There was no significant difference between the glucose content in the leaves of plants treated with DCMU and the plants treated with DBMIB, so glucose is not the only or the main factor that regulates expression of the studied genes. We presume that expression of Arabidopsis *GDH1* and *GDH2* genes depends on the chloroplast electron transport chain redox state. This regulatory mechanism could arise because of a need to avoid a competition for substrate between tetrapyrrole synthesis, glutathione synthesis and using of L-glutamate as an energy source during prolonged darkness.

Introduction

Light is one of the most important factors affecting development of a plant organism. Expression of 30 to 50% of plant genes changes when shifting from the dark to the light (Rasmusson and Escobar 2007). A significant proportion of light-dependent regulatory signals has a chloroplast origin. Chloroplast-to-nucleus signals regulate synthesis of photosynthetic electron transport chain components (Pfannschmidt et al. 2001; Borisova-Mubarakshina 2014), carotenoid biosynthesis (Chan et al. 2016), and expression of a number of antioxidant defense genes (Fey et al. 2005; Dietz et al. 2016). Although a large number of these signals are known, the molecular mechanisms of light-dependent regulation of gene expression remain unclear in many cases.

One of those cases is regulation of plant NAD-dependent glutamate dehydrogenase (GDH, EC 1.4.1.2) gene expression. This is a mitochondrial enzyme that catalyzes deamination of L-glutamate to 2-oxoglutarate (and the reverse reaction) and thus provides a link between carbon and nitrogen metabolism (Magadlela et al. 2019; Xiaochuang et al. 2020). GDH holoenzymes are hexamers consisting of two types of subunits, α and β, which are encoded in *Arabidopsis thaliana* by nuclear genes, respectively, *GDH1* (AT5G18170) and *GDH2* (AT5G07440). In addition, Arabidopsis has a *GDH3* gene (AT3G03910) encoding a minor γ subunit. It does not form a part of the holoenzyme and does not make a large contribution to total GDH activity in Arabidopsis leaves, although it may perform some fine regulation of GDH activity in other tissues and organs (Fontaine et al. 2013; Marchi et al. 2013). Transcript levels of *GDH1* and *GDH2*

genes are highly dependent on the light intensity: they are high in the dark and very low in the light (Turano et al. 1997; Lee et al. 2010).

2-Oxoglutarate formed by GDH activity can be metabolized in the TCA cycle to obtain ATP (Robinson et al. 1992; Labboun et al. 2009). Miyashita and Good (2008) demonstrated that Arabidopsis wild type plants survived extended darkness for longer than *gdh1* and *gdh2* knockout mutants. So, it was suggested that plant GDH can act as back-up in cases of carbohydrate starvation. When the glucose level is decreased, *GDH1* and *GDH2* genes are de-repressed, and *de novo* synthesized GDH enzyme allows the use of L-glutamate as a source of energy (Miyashita and Good 2008). This hypothesis is speculative and has been neither confirmed nor refuted experimentally. Soluble sugars do affect the expression of GDH genes. Transcript levels of *GDH1* and *GDH2* genes decreased 2–2.5 fold after treatment of 7-day-old Arabidopsis seedlings with a 3% glucose solution for 2–6 h (Li et al. 2006). However, GDH genes expression decreases by orders of magnitude when plants were transferred from the darkness to the light (Turano et al. 1997; Garnik et al. 2013). Such a decline could not be achieved by treatment with exogenous sugars. When studying the possible role of hexokinase 1 as a glucose sensor in the regulation of *GDH1* and *GDH2* genes expression, we also failed to achieve a significant decrease in expression of these genes by treating plants with exogenous sucrose (Garnik et al. 2014).

All the above suggests that the hypothesis of Miyashita and Good is not the only possible explanation for the light-dependent changes in the expression of GDH genes. In the present study, we show that expression of the *GDH1* and *GDH2* genes in Arabidopsis is regulated by redox signals of chloroplast origin that depend on the photosynthetic electron transport.

Materials And Methods

Plant material and experimental conditions

Seeds of *Arabidopsis thaliana* (L.) Heynh (ecotype Col-0) were obtained from the Arabidopsis Biological Resource Center (The Ohio State University, Columbus, Ohio, USA). Seeds were sterilized in 70% (v/v) ethanol with 0.05% (v/v) Triton X-100, rinsed thrice with sterile water and placed on solid medium in Petri plates along the diameter of the plate. The 0.5X Murashige and Skoog growth medium (Sigma-Aldrich, USA) with 0.8% Phytagel (Sigma-Aldrich) was used. After a stratification at + 4 °C for 3 days, the plates were mounted vertically and seedlings were grown for 14 days at 23 °C, a light intensity of 120 μmol * m^{-2} * s^{-1}, and 16 h : 8 h light : dark photoperiod. Fluorescent lamps Philips TL-D 36W / 54–765, color temperature 6200K, were used as a light source (white light). In experiments with photosynthetic electron transport inhibitors, all seedlings were kept in the dark for 18 h before the start of treatment. 3- (3.4-dichlorophenyl)-1.1-dimethylurea (DCMU), 2.5-dibromo-3-methyl-6-isopropyl benzoquinone (DBMIB) and hydrogen peroxide treatments were performed by spraying seedlings (leaves and roots) with solutions containing: DCMU (Sigma-Aldrich) – 10 μM, DBMIB (Sigma-Aldrich) – 100 μM, H_2O_2 – 10 mM. All solutions contained 0.01% Triton X-100. Deionized water containing appropriate concentrations of solvents and Triton X-100 was used as a mock treatment. After spraying with DCMU, DBMIB or hydrogen

peroxide, the plants were incubated either in the dark or at 120 μmol * m^{-2} * s^{-1} for 2 and 4 h, and then 8 seedlings from each group were taken for total RNA isolation. Each experiment was repeated three times at least.

Superoxide radical assay

The superoxide radical (O_2^-) in the leaves of 14-day-old plants was evaluated qualitatively by staining with nitro blue tetrazolium (Tarasenko et al. 2012). Before the start of experiment, plants were kept in the dark for 18 h. The detached leaves were infiltrated with 1 mg/ml solution of nitro blue tetrazolium (Thermo Scientific, USA) in 10 mM KH_2PO_4, pH 7.8, or with the same solution containing DCMU (10 μM) or DBMIB (100 μM). Then the leaves were kept in the dark or in the light (120 μmol * m^{-2} * s^{-1}) for 1 h, washed free from chlorophyll in 70% ethanol at 80 °C and photographed.

RNA extraction and Real-Time PCR (RT-PCR)

Total RNA was extracted from rosette leaves using TRI-Reagent (Ambion, USA) according to the manufacturer's protocol. A sample of 400 ng of total RNA pretreated with DNase I (Thermo Scientific) was used for cDNA synthesis. The synthesis was performed using random hexamer primers (Thermo Scientific) and RevertAid H Minus M-MuLV reverse transcriptase (Thermo Scientific) according to the manufacturer's protocol. The RT-PCR was performed using a SYBR Select Master Mix (Applied Biosystems, USA) and C1000 Thermal Cycler CFX96 Real-Time PCR Detection System (Bio-Rad, USA) according to the following protocol: heating to 50°C, 2 min, one denaturation cycle (95°C, 3 min), 36 amplification cycles (95°C, 20 s – 60°C, 30 s – 72°C, 30 s). The *YLS8* gene was used as a reference (Hong et al., 2010). The following primers were used: *YLS8* (At5g08290) – L: AGGTGCTTGCGTCTGTTGCT, R: TGTCCTTGAGAGCCCAGTTGAT; *GDH1* (At5g18170) – L: CAGGGCAGCGTTTTGTCATCCA, R: CGATACCATCCTTGTTCTTGATTGCT; *GDH2* (At5g07440) – L: CGCTCTTGGTGGTGTCCTGAA, R: CTCCTCCTGCGTTTGCGTAGA; *BAP1* (At3g61190) – L: GCGTGTTCGTCCCAAGCG, R: GCGTTGATACAGACCCCAAACC; *NOD1* (At5g64870) – L: ACGAGAAGCAGAAGCAAGCG, R: GGTAAGTCCCCTGAGCATCGG.

Glucose assay

Glucose assay was carried out by an enzymatic method using the Glucose (HK) Assay Kit (Sigma-Aldrich). Arabidopsis leaves (300 mg fresh weight) were homogenized in liquid nitrogen with a mortar and pestle. 600 μl of double-distilled water was added, and the samples incubated for 30 min at 80°C and centrifuged for 10 min at 15000 g. The supernatant was used to assay glucose levels according to the manufacturer's protocol, and the SmartSpec Plus spectrophotometer (Bio-Rad, USA) was used to measure the absorbance of the solutions at 340 nm. The glucose concentration in the supernatant was determined by the formula:

*glucose concentration, mg/mL = (ΔA*TV*F*180.2) / (6.22*d*SV*1000)*,

where *ΔA* is the difference in absorbance of the sample and the comparison solution; *TV* is the total sample volume, *mL*; *F* is the dilution factor; 180.2 - molar mass of glucose, *g/mol*; *SV* is the volume of supernatant in the sample, *mL*; 6.22 is the millimolar extinction coefficient for NADH at 340 nm; *d* is the optical path length (= 1 cm); 1000 - conversion coefficient μ*g* to *mg*. The final result was expressed in *mg* glucose per *gram* of fresh weight of Arabidopsis tissue.

Statistical analysis

The experiments were repeated three times or more. Leaves from 8 to 12 Arabidopsis plants were pooled into each sample. All experimental data were statistically analyzed using Student's t-Tests. Mean values ± standard deviation of the mean (SD) were calculated from the results of at least three replicates, and significant differences relative to controls are presented at * $P < 0.05$.

Results

To study dynamics of changes in *GDH1* and *GDH2* expression during the light shifts, 14-day-old Arabidopsis plants were adapted to 120 μmol * m^{-2} * s^{-1} of the white light for at least 18 hours, then placed in the dark, and leaf samples were taken every 2 h. Similarly, plants adapted to the dark were transferred to the white light (120 μmol * m^{-2} * s^{-1}) and leaf samples were taken after 0.5, 1, 2, 4 and 6 h. Decrease in *GDH1* and *GDH2* expression after the dark-to-light shift occurred rapidly. Within the first 2 h, transcript levels of the both genes reached low values and then decreased slightly (Fig. 1a). In contrast, the increase in the transcript levels after the light-to-dark shift developed slowly and did not reach a plateau during the 8 h incubation. Based on these data, in further experiments, we carried out adaptation of plants to the darkness before the start of experiments for at least 18 h.

To study dependence of *GDH1* and *GDH2* expression on light intensity, 14-day-old plants grown at 120 μmol * m^{-2} * s^{-1} of white light were adapted to experimental light conditions (0; 2.5; 5; 25; 40; 95 μmol * m^{-2} * s^{-1}) for two days. Time of continuous light exposition before sampling was 4 h. For both genes, higher expression values were observed at low and very low light intensity. At 25 μmol * m^{-2} * s^{-1} and higher, expression of both genes was less than 20% of the transcript levels in the dark. With a further increase in light intensity, the expression levels continued to decrease slowly (Fig. 1c). Thus, expression of both genes is very sensitive to light and decreases significantly even at the low light.

Due to the structural features of chloroplast electron transport chain (C-ETC), it is possible to block electron transfer using DCMU at the level of the Q_A-Q_B sites in the reaction center of PSII, or using DBMIB at the level of the Q_0 site of the cytochrome b_6/f complex. Under the moderate light conditions, a more oxidized state of plastoquinone pool is achieved if DCMU is applied, and a more reduced state in the case of DBMIB (Wang et al., 2016). To study the effect of C-ETC redox state on the expression of *GDH1* and *GDH2*, we treated *in vivo* 14-day-old Arabidopsis plants by DCMU or DBMIB and exposed them to 120 μmol * m^{-2} * s^{-1} of white light for 4 h (Fig. 2a). The transcript levels after DCMU treatment were significantly higher than in control plants: 8-fold for *GDH1* gene and 25-fold for *GDH2* (Fig. 2b). After

DBMIB treatment the transcript levels were slightly higher than in control plants, but significantly lower than after DCMU treatment.

In addition to the C-ETC redox state change, photosynthesis inhibitors can lead to changes in a glucose content as well as reactive oxygen species (ROS) generation. We analyzed glucose and O_2^- content in DCMU- and DBMIB-treated plants. After the treatment with both DCMU and DBMIB, the glucose level was significantly lower than in control plants (Fig. 2d). The O_2^- content in Arabidopsis leaves was significantly higher in the light than in the dark. After the DCMU treatment in the light, the O_2^- content was low, comparable to that in the dark. After the DBMIB treatment in the light, the O_2^- content was comparable with that in the control plants in the light (Fig. 2c).

One of the approaches to stimulate ROS generation in plants is high light exposure. In Arabidopsis, light intensities of more than 300 μmol * m^{-2} * s^{-1} result in elevated ROS generation and photooxidative stress (Boyes et al. 2001). In the next experiments we studied an effect of a high light on the *GDH1* and *GDH2* expression. 14-day-old plants grown as described earlier and adapted to 120 μmol * m^{-2} * s^{-1} of the white light were exposed to 900 μmol * m^{-2} * s^{-1} of the white light for 15 minutes, then RNA was isolated from the leaves immediately. *GDH1* and *GDH2* expression as well as $^1O_2$-responsive genes *BAP1* (At3g61190) and *NOD1* (At5g64870) (Saini et al., 2011) were evaluated. Induction of the *BAP1* and *NOD1* confirmed that the selected conditions led to a significant ROS generation (Fig. 3a). *GDH1* and *GDH2* transcript levels increased significantly after a 15-minute exposure under the high light (Fig. 3a).

We also studied an effect of exogenous hydrogen peroxide on the *GDH* genes expression. Arabidopsis plants were treated with hydrogen peroxide in two variants: in the dark and at 120 μmol * m^{-2} * s^{-1} of the white light. The initial *GDH1* transcript levels in the control plants adapted to the dark or the light differed very markedly (Fig. 3b). In the dark, treatment with H_2O_2 led to a slight increase in *GDH1* transcript content 2 and 4 h after the start of the treatment, and to a more pronounced *GDH2* expression increase (Fig. 3c). In the light, treatment with 10 mM H_2O_2 did not lead to any significant changes in *GDH1* expression, while *GDH2* transcripts content increased 2 h after the start of treatment and remained at the same level after 4 h (Fig. 3d).

Discussion

GDH1 and GDH2 expression depends on the intensity and duration of the light exposure

It is well known that *GDH1* and *GDH2* expression increases in the dark and decreases in the light (Turano et al. 1997; Lee at al. 2010), but dynamics of the expression changes during the light shifts has not been studied so far. In our experiments, the transcript levels of these genes in the dark are dozens more than in the light (Fig. 1, Fig. 3b). The decrease in *GDH1* and *GDH2* expression during the dark-to-light shift occurs more quickly than the increase during the light-to-dark shift (Fig. 1a, 1b). Comparison of the transcript content in plant leaves adapted to different light intensity provided the greatest differences in the range

from 0 to 25 μmol * m^{-2} * s^{-1} of the white light, *i.e.* at the low light. It suggests that a regulatory mechanism providing light dependent changes in the *GDH1* and *GDH2* expression is highly sensitive to light.

Earlier, we have demonstrated that functionally active chloroplasts are necessary for the light-dependent repression of *GDH1* and *GDH2* genes in Arabidopsis (Garnik et al. 2013). It means that the light-dependent regulation of *GDH1* and *GDH2* genes is mediated not by cytoplasm photoreceptors, but by plastid-to-nucleus signals.

DCMU treatment results in a higher expression of GDH1 **and** GDH2 **in the light**

Among a number of currently known plastid-to-nucleus signals, redox signals respond most quickly to the light intensity changes. This group includes signals mediated by singlet oxygen and Executer1 / Executer2 proteins; signals mediated by hydrogen peroxide generated in C-ETC; unknown signals associated with changes in the plastoquinone pool redox state (Dietz et al. 2016). Our attention was drawn to the latter regulatory path, since it is known that the pathway associated with Executer1 / Executer2 chloroplast-to-nucleus proteins is activated mainly under high light and photooxidative stress (Kim and Apel 2013), and hydrogen peroxide generation is closely associated with the plastoquinone pool redox state (Mubarakshina and Ivanov 2010). To manipulate with a plastoquinone pool redox state in plant leaves *in vivo*, we used the classical electron transfer inhibitors, DCMU and DBMIB.

DCMU treatment in the light led to the 8-fold higher transcript content for *GDH1* and 25-fold higher for *GDH2* (Fig. 2b). This can be interpreted as a participation of plastoquinone-derived redox signals in the regulation of *GDH* gene expression. This assumption is in a good agreement with a high sensitivity of the expression of the studied genes to differences in the light intensity under the low light conditions (Fig. 1c).

The redox state of the plastoquinone pool is important for the regulation of gene expression in the plastid (Pfannschmidt et al. 1999), as well as in the nucleus (Pfannschmidt et al. 2001). It is still not completely understood how such regulation is ensured in the cases where it is not mediated by ROS, but to date it has been observed for genes encoding the C-ETC subunits, the large Rubisco subunit (Pfannschmidt et al. 1999; 2001), chlorophyll-binding proteins of light-harvesting complexes (Borisova-Mubarakshina et al. 2014), flavonoid biosynthesis enzymes (Akhtar et al. 2010), and some antioxidant enzymes (Slesak et al. 2003; Yabuta et al. 2004; Garnik et al. 2016).

It was demonstrated using microarray technology that there are many more plastoquinone redox state-dependent genes. According to Adamiec et al., expression of 50 Arabidopsis genes (among 663 genes differentially expressed depending on the light intensity) returned to the dark levels after the DCMU treatment (Adamiec et al. 2008). A number of studies have shown participation of plastoquinone redox signals in responses of Arabidopsis transcript levels to stresses associated with high light and cold (Kopriva and Rennenberg 2004; Lepetit et al. 2013; Bode et al. 2016), as well as in regulation of stomata

closure (Wang et al. 2016). Thus, even processes not directly related to photosynthesis may depend on the plastoquinone pool redox state.

Changes in the expression of the GDH1 and GDH2 genes under the DBMIB treatment may be the result of its effect on mitochondrial ETC redox state

If we consider the redox state of the plastoquinone pool as the main factor influencing the expression of the *GDH1* and *GDH2* genes during light shifts, then we would expect that a DBMIB treatment will lead to an even greater decrease in their expression. However, the expression of the *GDH1* and *GDH2* genes under the DBMIB treatment was slightly higher than in the leaves of the untreated plants (Fig. 2b). The absence of a reduction can be explained by the rather high light intensity and, respectively, the low expression level of the studied genes in the control plants in the light.

On the other hand, it is known that in mitochondria DBMIB can bind to the *bc1* complex and inhibit the ubiquinol-cytochrome reductase activity, which leads to a reduced redox state of the mitochondrial ubiquinone pool (Surkov and Konstantinov 1980; Degli Esposti et al. 1984). We have shown previously that over-reduction of the ubiquinone pool by antimycin A treatment leads to induction of *GDH2* gene in Arabidopsis heterotrophic cell culture (Tarasenko et al. 2009). It is possible that DBMIB at the used concentration exerted an inhibitory effect on the mitochondrial ETC, which masked the effect of the plastoquinone pool redox state on the expression of the studied genes.

The lower glucose level in the plants treated with DCMU or DBMIB does not correlate with the changes in the GDH1 and GDH2 genes expression

The change in the plastoquinone pool redox state is not the only factor that can affect genes expression in the presence of DCMU or DBMIB. Each of these inhibitors blocks the light stage of photosynthesis and can lead to a decrease in glucose level in the cell. According to the Miyashita and Good hypothesis it is the level of soluble carbohydrates that is the main regulator of the glutamate dehydrogenase genes expression (Miyashita and Good 2008). Therefore, we estimated the glucose content in the leaves of the same plants in which the transcripts of *GDH* genes were determined.

After the treatment with DCMU or DBMIB, the glucose content in the leaves of plants become significantly lower than in the control. There was no significant difference between the glucose content in the leaves of plants treated with DCMU and the plants treated with DBMIB (Fig. 2d). Therefore, if in this experiment the transcripts levels depended mainly on the glucose content in the leaves, then the same increase in the *GDH1* and *GDH2* expression should be expected both after DCMU or DBMIB treatments. However, we observed the significantly higher transcripts levels after the DCMU treatment (Fig. 2b). It suggests that glucose is not the only and not the main factor that regulates the expression of the studied genes.

ROS generation cannot be a factor leading to a decrease in the GDH1 and GDH2 genes expression

ROS mediated signaling pathways take part in regulation of a huge number of plant genes (Dietz et al. 2016). The plastoquinone pool is one of the main ROS generation sites in the light, so treatment with

photosynthesis inhibitors significantly changes ROS level in plant cells, and it could be the reason for the observed *GDH1* and *GDH2* expression changes. The first ROS resulting from the leakage of electrons from a reduced or over-reduced plastoquinone pool is the singlet oxygen, which turns then into the superoxide radical O_2^- and the hydrogen peroxide H_2O_2 (Mubarakshina and Ivanov 2010).

We evaluated changes in the O_2^- content in Arabidopsis leaves after the DCMU or DBMIB treatment. In the presence of DBMIB, it was as high as in the light control. In the presence of DCMU in the light, it was much lower, comparable to the content in the dark (Fig. 2d). So, the lower level of superoxide radical corresponds to the higher level of *GDH1* and *GDH2* expression.

However, both our own results and the literature data clearly show that ROS can only increase the expression of glutamate dehydrogenase genes but not lower it (Scopelitis et al. 2006). In our experiments, when plants were exposed to 900 μmol * m^{-2} * s^{-1} of the white light, which is excessive for Arabidopsis and leads to photooxidative stress, the levels of *GDH1* and *GDH2* transcripts did not decrease, but increased (Fig. 3a). The treatment of plants with exogenous hydrogen peroxide also did not lead to a decrease in these genes expression neither at the initially high (in the dark), nor at the initially low (in the light) transcripts levels (Figs. 3c, 3d). This is in a good agreement with reports of increased expression of plant glutamate dehydrogenase during abiotic stresses. An increase in the GDH activity or in the expression of its genes during hypoxia-reoxygenation (Tsai et al. 2016), drought (Sun et al. 2013), temperature stresses (Goel and Singh 2015), and salt stress (Terce-Laforgue et al. 2015) was shown. Thus, ROS have an inducing effect on the *GDH* genes expression and cannot be the reason for a decrease in their expression during the dark-to-light shifts.

Physiological significance of the light-dependent regulation of the GDH1 **and** GDH2 **genes expression**

Turano et al. (1997) showed that the higher or lower *GDH1* and *GDH2* transcripts levels correlated with the higher or lower GDH enzyme activity, respectively. Thus, fluctuations in the transcript levels have a physiological significance.

We have here demonstrated that some chloroplast signals arising from changes in the C-ETC redox state can participate in the regulation of the *Arabidopsis thaliana GDH1* and *GDH2* genes expression. We believe that this regulation depends on the redox state of the plastoquinone pool, but is not mediated by ROS.

In the majority of the proven cases of gene expression regulation mediated by the plastoquinone pool redox state, the observed expression changes are aimed at adapting of the photosynthetic apparatus to changing light conditions, or at providing antioxidant protection. GDH, however, is a mitochondrial enzyme that is not directly related either to photosynthesis nor to other processes occurring in chloroplasts. However, the substrate of this enzyme, L-glutamate, also serves as a substrate for the biosynthesis of chlorophylls and heme groups of cytochromes belonging to the C-ETC (Tanaka et al. 2011).

L-glutamate is also necessary for synthesis of glutathione, which is necessary to provide antioxidant protection (Noctor et al. 2012). In plants, biosynthesis of both tetrapyrroles and glutathione is associated with chloroplasts and activated in the light (Tanaka et al. 2011; Noctor et al. 2012; Heyneke et al. 2013). Among the three metabolic pathways that use L-glutamate as the primary substrate (2-oxoglutarate formation; chlorophyll biosynthesis; glutathione biosynthesis), the second and the third are of a great importance in the light. On the other hand, the possibility of using L-glutamate in the TCA cycle after converting it to 2-oxoglutarate is important in cases of a long-term absence of photosynthesis (e.g. overnight).

We think that the physiological purpose of the light-dependent regulation of the *GDH1* and *GDH2* genes expression is to avoid a competition for the substrate between GDH (deamination of L-glutamate to 2-oxoglutarate), glutamyl-t-RNA synthetase (the first reaction of the tetrapyrroles biosynthesis path) and γ-glutamylcysteine synthetase (the first reaction of the glutathione biosynthesis path). In the light, expression of *GDH* genes is rapidly repressed, and L-glutamate can be used for biosynthesis of chlorophyll, heme and glutathione, which are more necessary at that time. After hours of absence of light, it becomes more necessary to use L-glutamate as a source of energy, that is ensured by increasing the expression of the *GDH* genes. The redox state of the C-ETC and the plastoquinone pool as a highly sensitive C-ETC component are ideal candidates to be the main regulators of light-dependent expression of the *GDH* genes.

Our hypothesis does not contradict the Miyashita and Good hypotheses about the participation of sugars in the light-dependent *GDH* regulation. However, Miyashita and Good suggested that the soluble sugars content should be the main regulatory factor for *GDH* genes expression changes, but we believe that the redox state of the plastoquinone pool is the factor playing the key role. Sugar accumulation as a *GDH* genes expression regulatory factor acts in the same direction as the plastoquinone pool reduction, but apparently makes a smaller contribution to their light-dependent repression.

Interestingly, glucose accumulation in the light itself can lead to a partial plastoquinone pool reduction due to a slowdown in the NADPH oxidation in the Calvin cycle (Ma et al. 2008). In this case, the decrease in the *GDH* genes expression in the presence of exogenous sucrose or glucose in the light can be explained not only by their direct regulatory action, but also by the glucose-dependent change in the plastoquinone redox state.

We have previously demonstrated that the expression of the *GDH2* gene is induced under the over-reduced state of the mitochondrial ubiquinone pool in a heterotrophic culture of Arabidopsis cells, and this induction was independent of ROS production (Tarasenko et al. 2009). There are many parallels in the structure and functioning of the respiratory and photosynthetic ETCs, and it is even more interesting that the reduction of the ubiquinone pool leads to the induction of *GDH* genes, and the reduction of the plastoquinone pool leads to their repression. It cannot be excluded that these are manifestations of the same regulatory mechanism mediated by redox signals emanating from the two largest quinone pools of a plant cell.

Declarations

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Human and Animal Rights

This research did not involve experiments with human or animal participants.

Informed Consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Author contributions

Conceptualization, Methodology, Writing - original draft preparation: Elena Yu. Garnik; Investigation, Validation, Data curation: Vadim I. Belkov; Resourses, Writing - reviewing and editing: Vladislav I. Tarasenko; Project administration: Yury M. Konstantinov. All authors discussed the results and approved the final manuscript.

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Figures

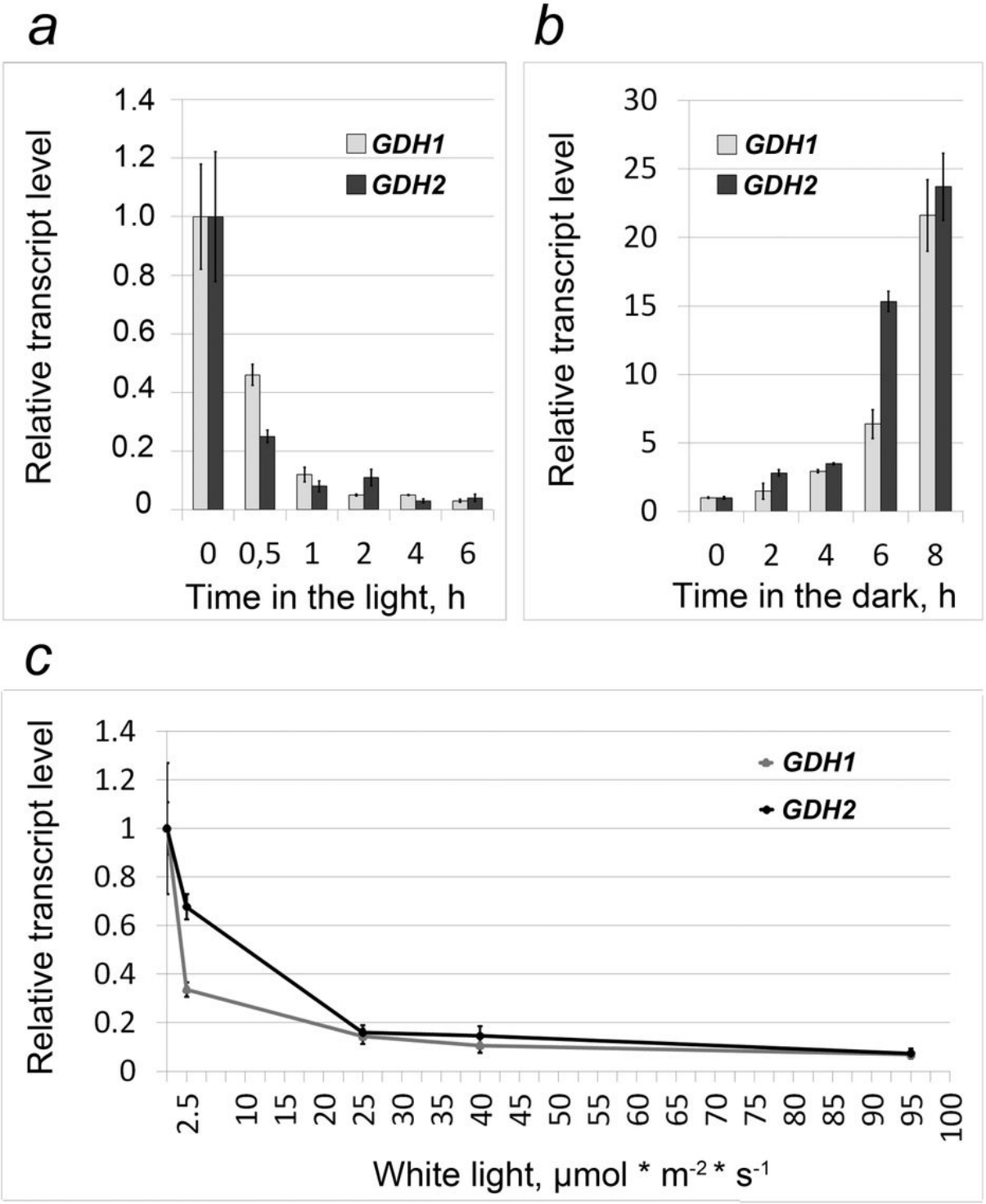

Figure 1

GDH1 and GDH2 genes transcript levels during the light shifts and under the different light intensities. a, plants were transferred from the dark to the light (120 µmol * m−2 * s−1). b, plants were transferred from the light (120 µmol * m−2 * s−1) to the dark. c, plants were adapted to 2.5; 5, 25; 40; 95 µmol * m−2 * s−1 of the white light for 2 days. The transcript levels at the beginning of each experiment (a, b) or in the dark (c) are taken as 1.

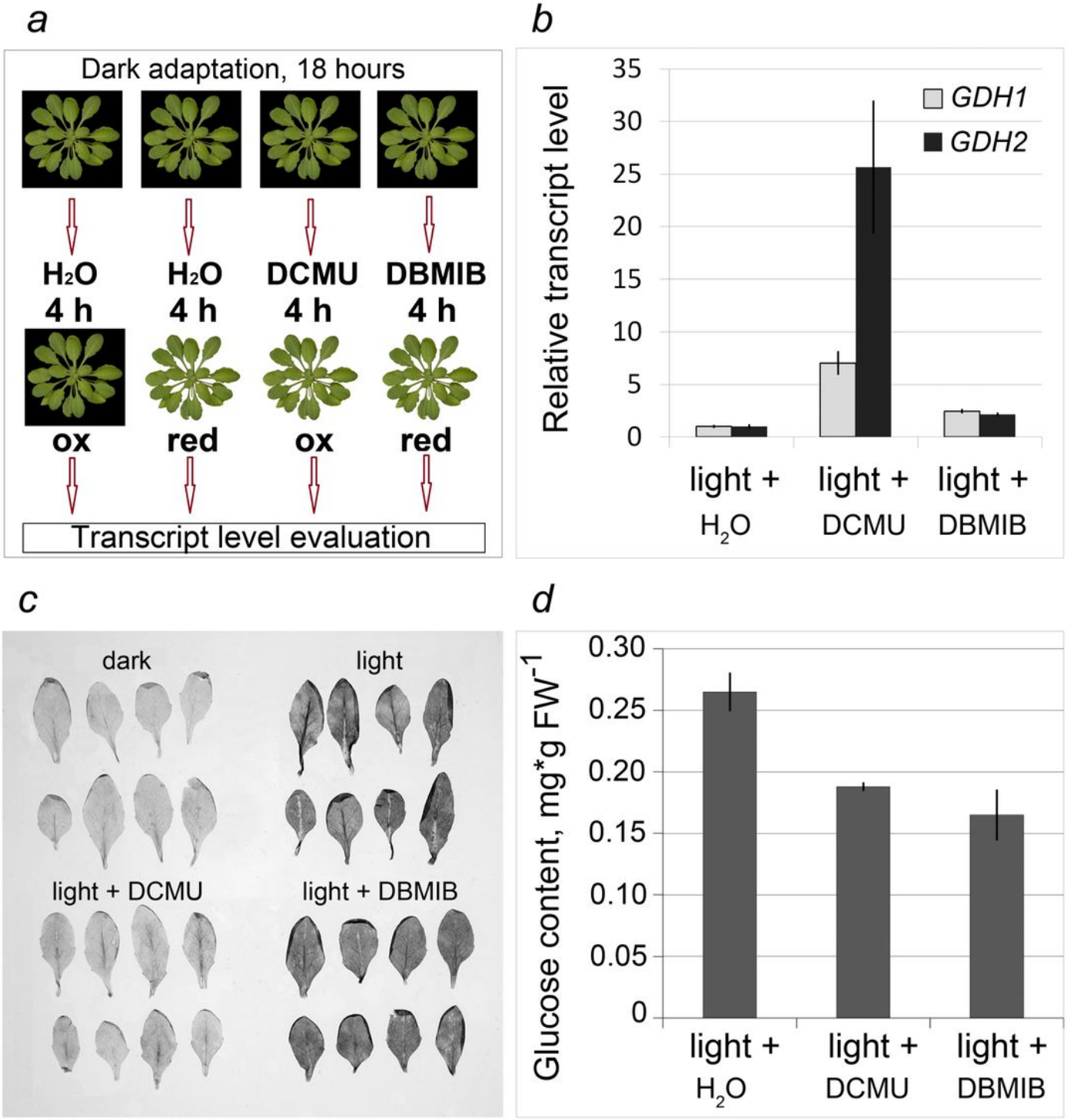

Figure 2

Effect of DCMU or DBMIB on the expression of GDH1 and GDH2 genes, O2– content and glucose content in leaves. a, an experiment design and expected redox states of the plastoquinone pool (PQ, “red” — reduced, “ox” — oxidized). b, the GDH1 and GDH2 transcript levels after the DCMU or DBMIB treatment. The transcript levels in control were taken as 1. c, O2– content in the leaves infiltrated with DCMU or

DBMIB. d, Glucose content in the leaves of plants treated according to scheme 2a. The * indicates differences with control at p <0.05.

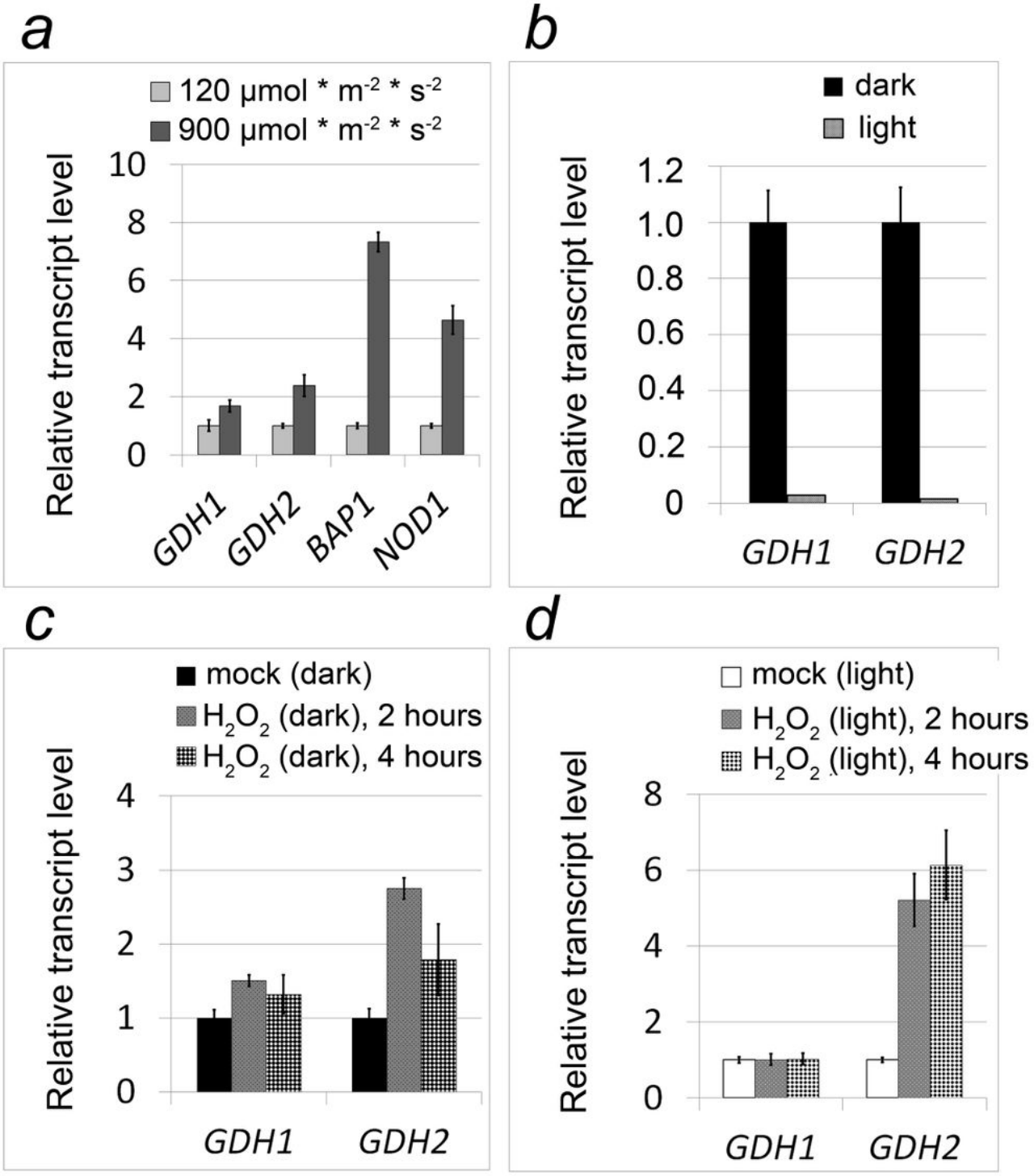

Figure 3

The effect of high light and exogenous H2O2 on the expression of GDH1 and GDH2 genes. a, plants were exposed at 900 μmol * m–2 * s–1 of the white light for 15 min, control - 120 μmol * m–2 * s–1. b, the transcript levels GDH1 and GDH2 in the plant leaves adapted to the darkness or the light (120 μmol * m–

2 * s−1). c, plants adapted to the darkness were treated with 10 mM H2O2 for 2 h and 4 h. Mock − the same plants were treated with H2O for 2 h. d, plants adapted to 120 µmol * m−2 * s−1 of the white light (control) were treated with 10 mM H2O2 for 2 h and 4 h. Mock − the same plants were treated with H2O for 2 h.