Journal of Plant Physiology 167 (2010) 540–546

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Extracellular ATP, nitric oxide and superoxide act coordinately to regulate hypocotyl growth in etiolated *Arabidopsis* seedlings

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ARTICLE INFO

Article history:
Received 23 July 2009
Received in revised form
3 November 2009
Accepted 4 November 2009

Keywords:
Arabidopsis thaliana
Extracellular ATP
Glutathione
Hypocotyl elongation
Nitric oxide

ABSTRACT

Etiolated *Arabidopsis thaliana* seedlings germinated in the presence of reducing buffers such as reduced gluthathione (GSH) and dithiothreitol (DTT) have altered morphology. GSH and DTT inhibited hypocotyl elongation in a dose-dependent manner. The GSH-mediated effect was prevented by the simultaneous addition of extracellular ATP (eATP). NADPH oxidase (NOX) activity and endogenous nitric oxide (NO) generation were required to mediate eATP action on the hypocotyl elongation. A correlation was observed between hypocotyl length, eATP concentration and NO production. The action of eATP and NO on superoxide ($O_2^{\bullet -}$) accumulation and peroxidase activity was investigated. The $O_2^{\bullet -}$ distribution was regulated by eATP and NO during hypocotyl elongation. Our data suggest that a finely tuned balance of redox status and optimal levels of ATP and NO are essential to regulate the hypocotyl elongation in the dark.

Introduction

Extracellular adenosine triphosphate (eATP) is a well established extracellular signal in a number of animal cell responses (Burnstock, 2006). In plants, eATP participates in many physiological responses such as cell viability (Chivasa et al., 2005), suppression of root growth (Tang et al., 2003), pollen germination (Steinebrunner et al., 2003) and pollen tube elongation (Roux and Steinebrunner, 2007). ATP can be released from plant cells from active growing tissue by plasma membrane ABC transporters (Thomas et al., 2000), vesicular efflux or wounding (Jeter et al., 2004). Evidences mainly based on the effects evoked by exogenous ATP applied to the plant cells pointed out the molecular mechanism of the eATP signaling pathway (Demidchik et al., 2003). In animals, eATP participates in signaling transmission via the activation of P2X and P2Y purinoreceptors (Ralevic and Burnstock, 1998). Presence of purinoreceptor homologs for extracellular nucleotides in plants have not been demonstrated yet. However, high similarities have been described between plant and animal responses to eATP perception (Clark and Roux, 2009). In plants, ATP increases Ca^{2+} influx in the cytoplasm and triggers superoxide ($O_2^{\bullet -}$) production by NADPH oxidase (NOX) activity coupled to downstream gene expression changes (Song et al., 2006). Demidchik et al. (2009) demonstrated that eATP causes the production of reactive oxygen species (ROS) in intact roots through the plasma membrane NOX, suggesting that in plants, an eATP signaling by NOX and Ca^{2+} channels could have evolved as a distinct mechanism to transduce the eATP signal. Nitric oxide (NO) production stimulated by eATP was demonstrated in tomato suspension cells (Foresi et al., 2007) and hairy roots of *Salvia multiorriza* (Wu et al., 2008). In algae, Torres et al. (2008) also described eATP-mediated NO production. The suppression of pollen germination and pollen tube elongation is also mediated by eATP and NO signaling pathway (Reichler et al., 2009). However, it is still unknown how eATP and NO interact to mediate plant growth responses.

Investigations on plant growth and developmental responses have showed the convergence of a set of regulatory signaling pathways. A notable example is the hypocotyl growth in etiolated seedlings. In *Arabidopsis* eATP signaling pathway modulates hypocotyl growth of etiolated seedlings; concentrations of non-hydrolyzable ATP analog (ATP-γ-S) in the range 100–200 μM promote the hypocotyl growth, while doses above 400 μM inhibit this process (Roux et al., 2006; Roux and Steinebrunner, 2007). The addition of a NO donor to etiolated *Arabidopsis* seedlings promotes de-etiolation and inhibits the hypocotyl elongation

Abbreviations: ATP-γ-S, non-hydrolyzable ATP analog; AU, arbitrary units; cPTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; DAF-FM DA, 3-amino, 4-aminomethyl-2,7-difluorofluorescein diacetate; DPI, diphenyleneiodonium chloride; DTT, dithiothreitol; eATP, extracellular adenosine triphosphate; GSH, reduced glutathione; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOX, NADPH oxidase; $O_2^{\bullet -}$, superoxide; PPADS, pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate

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doi:10.1016/j.jplph.2009.11.002

(Beligni and Lamattina, 2000). Reducing agents such as ascorbate and reduced glutathione (GSH, γ-glutamylcysteinyl glycine) decrease the hypocotyl growth of etiolated lupin seedlings (Cano et al., 1996). Cellular redox (reduction–oxidation) systems influence different processes in animals and plants. The control of redox homeostasis implies a variety of signal transduction pathways and metabolic adjustments (Day and Suzuki, 2005).

The involvement of eATP, NO and GSH in the same physiological process led us to explore whether these molecules act co-ordinately to modulate the growth of etiolated hypocotyls. We showed that young and actively growing tissues were very sensitive to redox homeostasis. In addition, we describe the concurrence of redox status, eATP and NO signaling pathways to modulate the hypocotyl elongation in darkness.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh ecotype Columbia (*A. thaliana*) were used. Seeds were surface sterilized by soaking them in 30% sodium hypochlorite and 0.02% Triton X-100 for 15 min and then washed extensively in sterile water. Seeds were stratified by placing them for 3 d at 4 °C in the dark. To promote and synchronize the germination a 30 min pulse of red light (20 μmol photons s^{-1} m^{-2}) was applied before placing the seeds on petri dishes containing ATS medium and 0.8% agar (Beligni and Lamattina, 2000) since germination of *A. thaliana* seeds is under the control of different phytochromes (Casal et al., 1998). Plates were orientated vertically in a growth chamber for 1 or 3 d at 25 °C in the dark.

Pharmacological treatments

Dithiothreitol (DTT) and GSH were used as reductants. ATP stock solution was adjusted to pH 5.7 and then used. Adenosine-5′-(γ-thio)triphosphate tetra-lithium salt (ATP-γ-S) as an agonist, and suramin (8-[3-benzamido-4-methylbenzamido]-naphtalene-1,3,5-trisulfonic acid) and pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS) from Sigma-Aldrich, St. Louis, MO, USA, as non-selective and selective antagonists of P2 receptors, respectively, were used. Diphenyleneiodonium chloride (DPI) from Sigma as a NOX inhibitor was tested. Potato apyrase enzyme (EC 232-569-8, Sigma-Aldrich, St. Louis, MO, USA) at concentrations of 20, 80 and 200 U mL^{-1} was added to ATS agar medium. The specific NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO; Invitrogen Molecular Probes, Eugene, OR, USA), 100 μM N^G-nitro-L-arginine methyl ester (L-NAME; Calbiochem) and 1 mM tungstate were also tested.

Quantification of hypocotyl lengths

To measure the hypocotyl length, 3-d-old *A. thaliana* seedlings grown under different treatments were photographed. Lengths were measured using ImageJ 1.33 software (National Institute of Health, USA). Four independent experiments were performed and at least 20 seeds were compared in each treatment.

Measurement of NO production

A. thaliana seeds were germinated in solid ATS medium supplemented with indicated compounds and grown as indicated above. For determination of NO production, seedlings were incubated in 5 μM of the cell-permeable fluorescent probe 3-amino, 4-aminomethyl-2,7-difluorofluorescein diacetate (DAF-FM DA; Calbiochem, San Diego, CA, USA) in 20 mM Hepes–NaOH pH 7.5 for 30 min. Thereafter, seedlings were washed three times with fresh buffer and examined by epi-fluorescence (DAF-FM DA excitation 490 nm, emission 525 nm) in an Eclipse E 200 microscope (Nikon, Tokyo) connected with a high resolution digital camera (Nikon). Quantification of fluorescence from images acquired from the microscope was achieved in selected areas of equal size and measurements of the average pixel intensity were estimated with the ImageJ 1.33 software (National Institutes of Health, USA).

$O_2^{\bullet -}$ production

A. thaliana seeds were germinated in solid ATS medium supplemented with different compounds for 48 h in darkness. Seedlings were stained for 60 min in a solution containing 0.2% nitroblue tetrazolium (NBT) in 10 mM phosphate buffer at pH 8.0, NBT forms a dark blue/violet formazan precipitate in contact with $O_2^{\bullet -}$. The reaction was stopped by placing seedlings in distilled water. Seedlings were observed under a Nikon SMZ800 magnifier equipped with a Cool SNAP-Pro Cf color (Roper Scientific) camera or under an Eclipse E 200 microscope (Nikon, Tokyo) connected with a high resolution digital camera (Nikon).

Peroxidase activity

Two-day-old seedlings grown in the dark were stained with a solution containing 1 mM *o*-dianisidine and 10 mM H_2O_2 for 60 min and then washed in water. Pictures were taken as described for NBT staining.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and post hoc comparisons were done with Tukey's multiple range test to determine statistical significance among treatments at $P < 0.05$ level. The statistical software program used was SigmaStat 3.1.

Results

Exogenous ATP restores redox-mediated disruption of the hypocotyl growth in darkness.

Etiolated seedlings germinated in the presence of GSH have altered morphology. Fig. 1A shows that 3-d-old GSH-treated seedlings evidenced a remarkable inhibition of hypocotyl elongation with no open cotyledons, and with a clear loss of the negative shoot gravitropism. Quantification of the hypocotyl length at 3 d after treatments indicated that GSH-treated seedlings exhibited hypocotyls approximately 4 fold shorter than control (Fig. 1B). GSH as well as DTT inhibited hypocotyl elongation in a dose-dependent manner (Supplemental Fig. S1A). The addition of exogenous ATP (0.1, 0.2, 0.5 and 1 mM) restored the GSH-mediated disruption of the hypocotyl elongation in a dose-dependent manner (Fig. 1B). Similar effects had the addition of exogenous ATP on DTT-treated seedlings (Supplemental Fig. 1B). ATP-γ-S acts as an agonist of P2 receptors mimicking ATP effects at lower concentrations (Tang et al., 2003). Addition of ATP-γ-S at 0.2 and 0.3 mM also reversed the GSH-mediated inhibition of hypocotyl growth (Fig. 1C). Suramin, a broad-spectrum

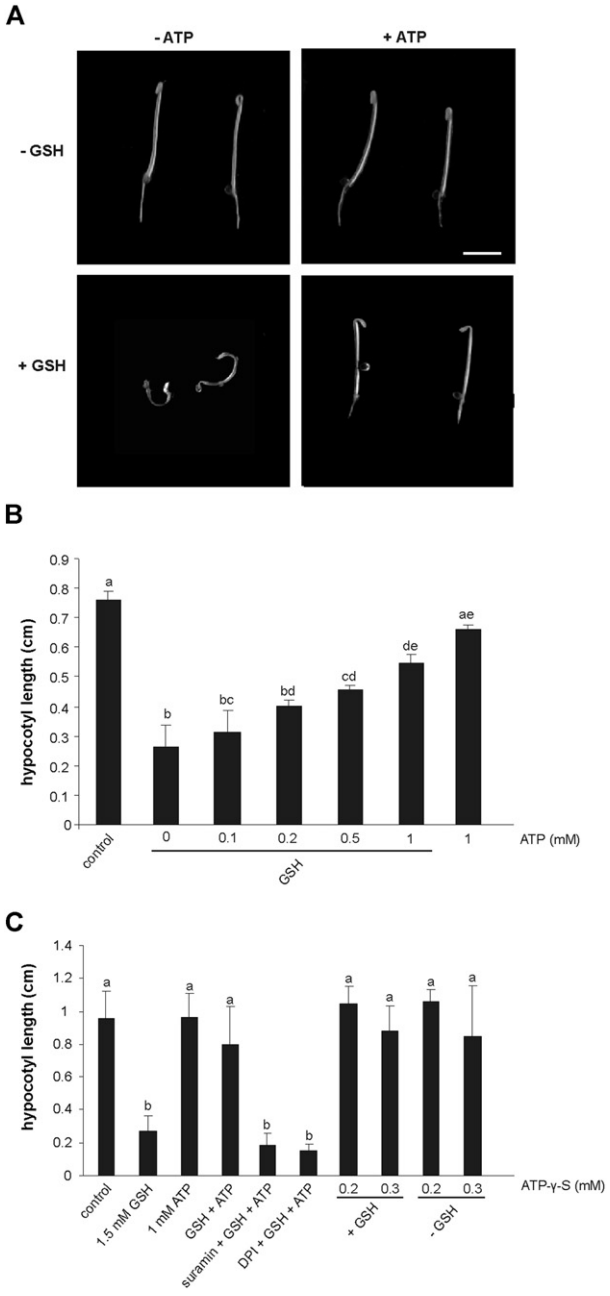

Fig. 1. eATP restores the normal hypocotyl elongation of GSH-treated seedlings in darkness. (A) Representative 3-d-old seedlings grown in ATS medium containing 1 mM ATP, 1.5 mM GSH or GSH+ATP. Bar=4 mm. (B) Measurements of hypocotyl lengths grown in the absence or presence of 1.5 mM GSH or ATP (0.1, 0.2, 0.5 and 1 mM). Combinatorial treatments are indicated. (C) Measurements of hypocotyl lengths from seedlings grown in ATS containing 1.5 mM GSH, 1 mM ATP, 0.1 mM suramin, 0.01 mM DPI or ATP-γ-S (0.2 and 0.3 mM). Twenty seedlings per treatment were analyzed in each experiment. Data shown are the mean (± SD) of three independent experiments. Different letters indicate significantly different means between treatments ($P < 0.05$).

antagonist of P2 receptor, and DPI (a NOX inhibitor) reduced the ability of ATP to restore hypocotyl growth in GSH-treated seedlings (Fig. 1C). The effect of exogenous ATP on hypocotyl elongation gradually decreased as concentrations of the specific antagonist of purinoreceptors, PPADS, increased (Supplemental Fig. S2). These findings indicate that the ATP signal is transduced via P2 purinogenic-like receptors in etiolated hypocotyl. NOX activity is also required.

NO mediates the eATP effect restoring the hypocotyl elongation in GHS-treated seedlings in darkness.

We hypothesized that eATP and NO may act coordinately to modulate the hypocotyl elongation in etiolated *Arabidopsis thaliana* seedlings. To examine the involvement of endogenous NO in the eATP-mediated action, the NO-specific scavenger cPTIO was used. Seedlings were treated with GSH, ATP or cPTIO and their combination. The hypocotyl length decreased in both GSH plus ATP and in non-GSH-treated seedlings in the presence of 0.5 and 1 mM cPTIO (Fig. 2A). These data indicate that NO mediates the eATP action on the hypocotyl elongation. Similar results were obtained when nitrate reductase (NR) and NO synthase, two enzymatic activities involved in NO production, were inhibited. Tungstate, a NR inhibitor, and L-NAME, a NO synthase inhibitor, partially reduced the hypocotyl elongation in the presence of GSH plus ATP (Fig. 2B) as well as in the absence of GSH (data not shown).

To investigate the effect of ATP-γ-S addition on the NO level, seedlings were loaded with the NO-specific fluorophore DAF-FM DA and photographed under fluorescence microscope (Fig. 3A). The quantification of fluorescence indicated that NO production clearly increased by treating seedlings with 0.8 mM ATP-γ-S (Fig. 3B). In agreement with Roux et al. (2006) we observed a gradual decrease of the hypocotyl length for doses above 0.3 mM ATP-γ-S (data not shown). cPTIO diminished the ATP-γ-S-induced fluorescence, confirming that it is caused by NO production (Fig. 3A, B). In addition, NO production was measured in 2d-old seedlings grown in ATS medium containing GSH or GSH supplemented with different concentrations of ATP-γ-S. Again, coincidentally short hypocotyls of 1.5 mM GSH-treated seedlings showed the highest level of NO. Strikingly, as the ATP-γ-S

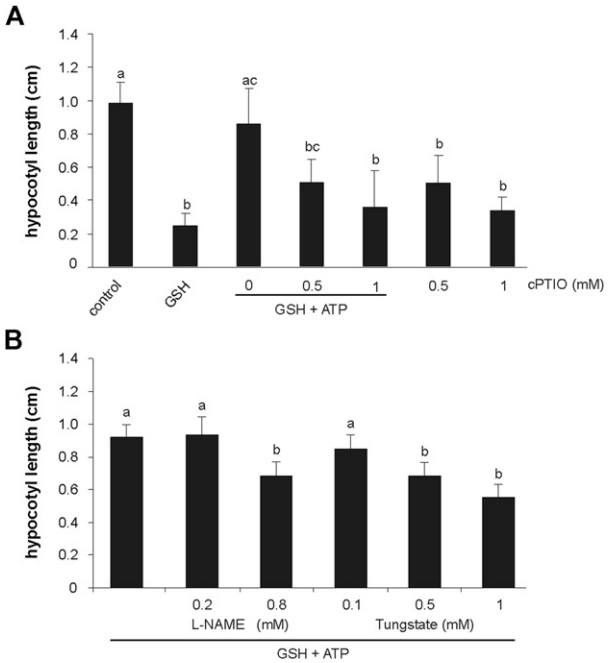

Fig. 2. Nitric oxide (NO) is required for eATP action in non-treated and GSH-treated seedlings. Measurements of hypocotyl length of 3-d-old seedlings. (A) Seedlings were grown in the absence or presence of 1.5 mM GSH, GSH plus 1 mM ATP and the specific NO scavenger cPTIO (0.5 and 1 mM). Combinatorial treatments are indicated. (B) GSH plus ATP-treated seedlings were grown in the absence or presence of L-NAME and tungstate. Twenty seedlings per treatment were analyzed in each experiment. Data shown are the mean (± SD) of at least two independent experiments. Different letters indicate significantly different means between treatments ($P < 0.05$).

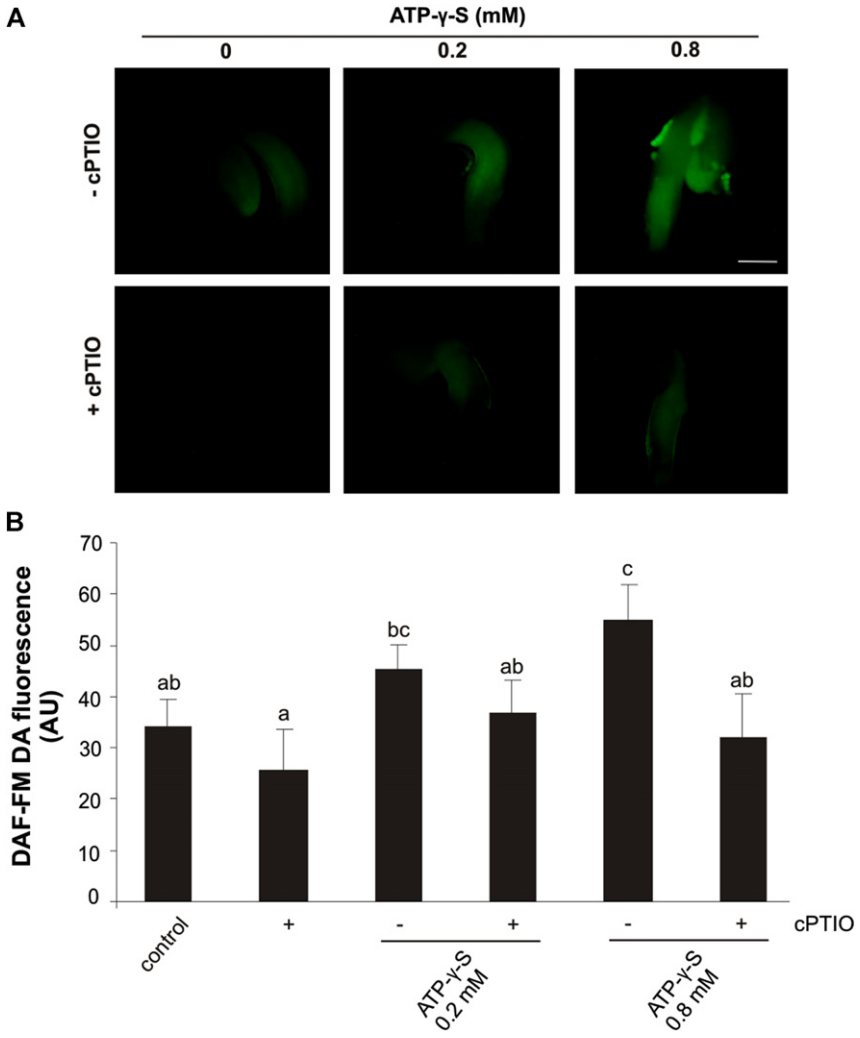

Fig. 3. eATP restores NO homeostasis in GSH-treated hypocotyls of etiolated seedlings. NO fluorescence was measured with the specific probe DAF-FM DA. (A) NO fluorescence detected in 1-d-old seedlings grown in the absence or presence of 1 mM cPTIO, ATP-γ-S (0.2 and 0.8 mM) or their combination in darkness. Bar=0.25 mm. (B) NO production was quantified and expressed as arbitrary units (AU). Bar=0.2 mm. Ten seedlings per treatment were analyzed in each experiment. Data shown are the mean (± SD) of three independent experiments. Different letters indicate significantly different means between treatments ($P < 0.05$).

concentration increased, NO production gradually decreased in GSH-treated seedlings (Fig. 4A, B). ATP had a similar effect to that of ATP-γ-S on NO production in GSH-treated seedlings but ATP-γ-S mimicked ATP effects at lower concentrations (Fig. 4C, D). Interestingly, NO level was rather similar between 1 mM ATP and control (Fig. 4C). Thus, as long as the concentration of exogenous ATP-γ-S or ATP is below the level needed to induce a significant accumulation of NO, it does not interfere with hypocotyl elongation. However levels of eATP or ATP-γ-S high enough to induce a significant NO accumulation can inhibit hypocotyl elongation. Additionally, treatment with the NO scavenger cPTIO that reduces the NO concentration below a threshold level to promote hypocotyl elongation results in hypocotyl growth inhibition (Fig. 2A). Therefore, we suggest that a fine tuning balance of redox status and optimal levels of ATP and NO are essential to regulate the hypocotyl elongation in darkness.

$O_2^{\bullet-}$ distribution correlates with changes of NO accumulation in the A. thaliana hypocotyl

Since redox status, ATP and NO seem to be operating in the hypocotyl elongation control we examined the $O_2^{\bullet-}$ distribution by using NBT. In elongated hypocotyls of 3-d-old seedlings $O_2^{\bullet-}$ distribution had a typical pattern (Fig. 5A, upper, right and left panels). A gradient of dark blue/violet stained cells was observed from the base toward the tip of the hypocotyl. The gradient denoting the presence of $O_2^{\bullet-}$ was expanded to the half of the hypocotyl axis next to the base. As a result of GSH treatment, hypocotyls showed a dark area at their bases and several dark spots irregularly distributed toward the tip (Fig. 5A, lower left panel). Inserts in Fig. 5A show in more detail these differences between GSH-treated and non-GSH-treated seedlings. The addition of exogenous ATP restored the pattern of $O_2^{\bullet-}$ distribution (Fig. 5A, lower right panel). As the rate of endogenous eATP accumulation and depletion is controlled by ectoapyrases, the addition of apyrase enzyme was also assayed in 2-d-old *A. thaliana* seedlings. The hypocotyls from seedlings grown in the presence of apyrase showed a decrease of their length values associated with a perturbed pattern of $O_2^{\bullet-}$ distribution (Supplemental Fig. 3S). A similar effect was also observed on cPTIO treatments (data not shown).

These findings highlight a relationship between the length of the hypocotyls and the alteration of $O_2^{\bullet-}$ distribution together with the ATP and NO availability. These results prompted us to analyze the peroxidase activity distribution in hypocotyls subjected to the same experimental conditions. The enzymatic activity was visualized by incubating seedlings in a solution

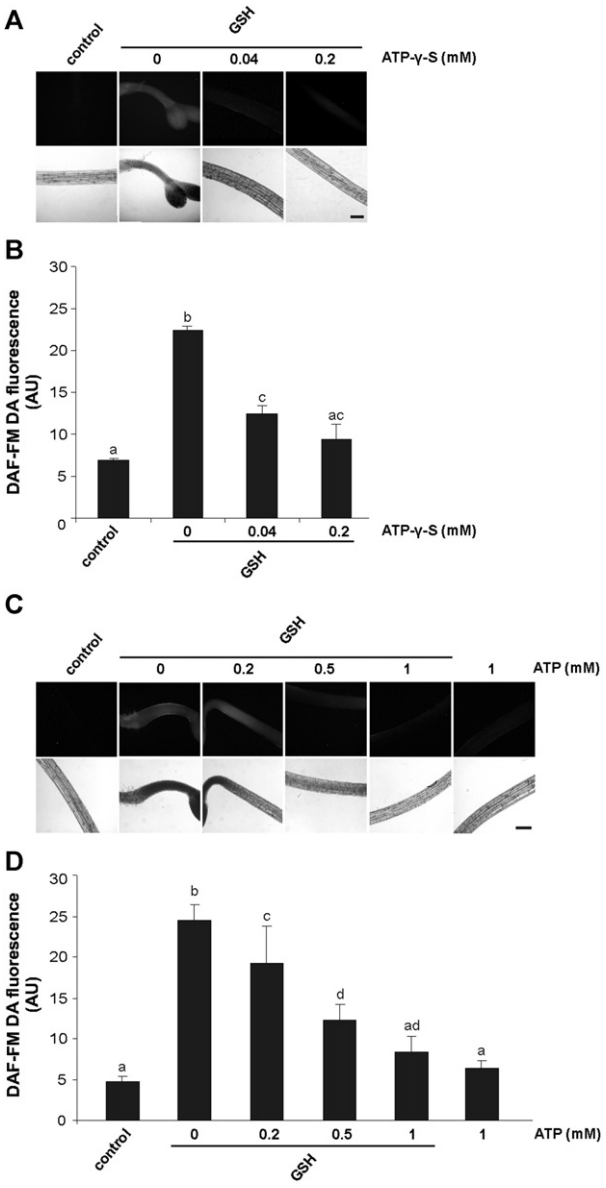

Fig. 4. eATP-γ-S modulates NO level in GSH-treated seedlings. (A) NO fluorescence detected in 2-d-old seedlings grown in the absence or presence of 1.5 mM GSH, ATP-γ-S (0.04 or 0.2 mM) or their combination (upper panel). Bright field (lower panel). (B) NO production was quantified and expressed as AU as indicated in (A). (C) NO fluorescence detected in 2-d-old seedlings grown in the ATS or ATS containing 1.5 mM GSH, GSH plus ATP (0.2, 0.5 or 1 mM) or 1 mM ATP (upper panel). Bright field (lower panel). (D) NO production was quantified and expressed as AU as indicated in (C). Ten seedlings per treatment were analyzed in each experiment. Data shown are the mean (± SD) of at least two independent experiments. Different letters indicate significantly different means between treatments ($P < 0.05$). Bars = 0.2 mm.

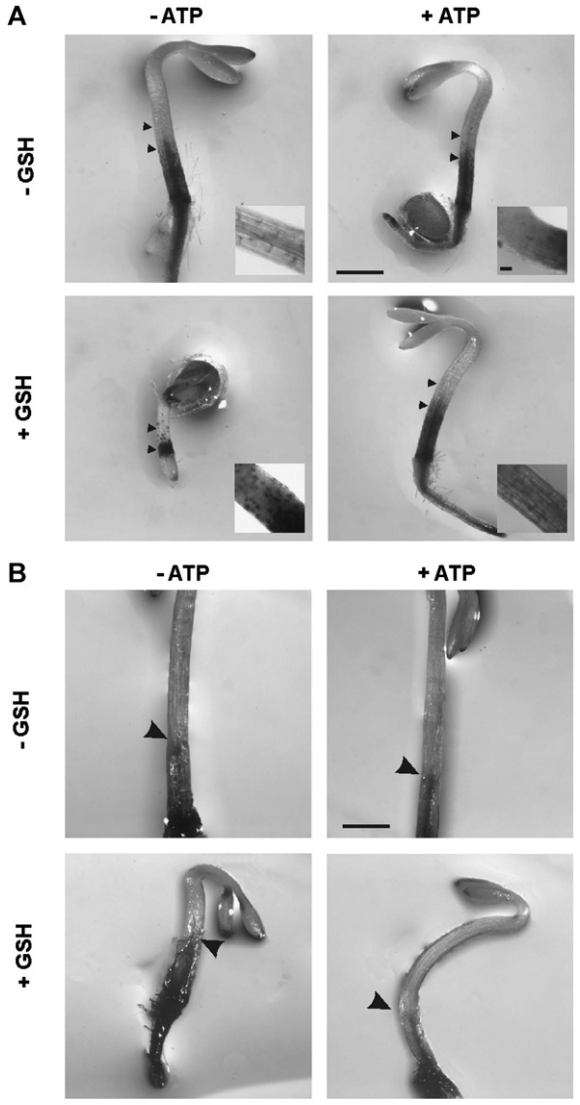

Fig. 5. Effect of eATP on $O_2^{\cdot -}$ production and peroxidase activity in hypocotyls (A). Two-day-old seedlings were grown in the absence or presence of 1 mM ATP, 1.5 mM GSH or GSH + ATP in darkness. Then, seedlings were stained with NBT and photographed under magnifier. The arrowheads indicate the hypocotyl zones that are magnified in inserts. Bar=0.5 mm Representative hypocotyls were photographed under microscope (inserts). Bar=0.05 mm. (B) Two-day-old seedlings grown under different combinatorial conditions were stained for peroxidase activity with *o*-dianisidine/H_2O_2 for 60 min. Photographs show representative seedlings assayed under each condition. The arrowheads indicate the interface between stained and non-stained zones of hypocotyl axis. Bar=0.5 mm.

containing *o*-dianisidine as hydrogen donor and H_2O_2. Under these conditions, the distribution of peroxidase activity visualized as a dark area was rather similar (Fig. 5B). As a control, seedlings were incubated in the presence of *o*-dianisidine without H_2O_2. No staining was observed (data not shown).

Discussion

A redox-associated regulation on an early morphogenetic process was analyzed in etiolated *A. thaliana* seedlings. Particularly, we focused on the action of reducing agents, such as GSH and DTT, on the hypocotyl elongation. Hypocotyl growth inhibition by redox state through GSH or DTT suggests that balanced pools of reductants/oxidants are essential for the hypocotyl elongation and negative gravitropism in etiolated *A. thaliana* seedlings. Different factors have been proposed as initiators of redox signal transduction pathways leading to changes in gene expression. H_2O_2, oxygen radicals, NO or soluble redox components such as GSH, and ascorbate could act as signals (Laloi et al., 2004; Foyer and Noctor, 2005; Ogawa, 2005). Light is one of the most important environmental factors regulating numerous plant developmental programs. Light induces photomorphogenesis by inhibiting hypocotyl elongation, cotyledon opening and light-responsive gene expression. *Arabidopsis* hypocotyls exhibit negative gravitropism when grown in the dark; however, the direction of growth is highly randomized when seedlings are exposed to continuous red light (Robson and Smith, 1996).

The phytochromes are a family of photoreceptors that controls plant responses to red and far-red light. Thus, the overlapping functions of red light and GSH in the regulation of hypocotyl growth could imply a biochemical action of such reducing agent on the phytochromes and/or signaling intermediates that act to transduce light perception in the hypocotyl cell.

We also demonstrated that GSH-mediated effects were prevented by eATP pathway throughout P2-like receptor and NOX activity in darkness. In parallel, NO depletion analysis using the NO scavenger cPTIO, NO synthase and NR enzyme inhibitors (L-NAME and tungstate, respectively), allowed us to assess the role of endogenous NO on hypocotyl growth. Removal of NO with cPTIO or inhibition of NO synthase and NR activities reduced the hypocotyl elongation, indicating that NO is required for the eATP-mediated hypocotyl growth. In addition, the pattern of $O_2^{\bullet -}$ distribution was finely regulated by eATP and NO. Taking together all these available evidence we suggest that a fine tuning of redox balance and NO production are involved in eATP effects. During the hypocotyl elongation of etiolated seedlings a precise $O_2^{\bullet -}$ distribution seems to play a prominent role. As was previously reported for *A. thaliana* roots, $O_2^{\bullet -}$ may participate in cell elongation (Dunand and Penel, 2007). Postembryonic growth of the *A. thaliana* hypocotyl is exclusively the result of controlled series of cell-elongation events. In the dark, cell elongation after germination is initiated only at the base and then the growing zone moved up the hypocotyl. However, this process is absent in light (Gendreau et al., 1997). Coincidently with the light effect, GSH caused a perturbation in the $O_2^{\bullet -}$ distribution in the hypocotyl cells. However, such disturbance was prevented by the addition of eATP. Thus, we suggest that eATP and NO signaling pathways control some components of redox status and NOX activity to regulate the cell elongation in the dark. Song et al. (2006) proposed a speculative model for the induction of $O_2^{\bullet -}$ production by eATP and its control by NOX homologs in *A. thaliana*. Demidchik et al. (2009) demonstrated that application of ATP to *A. thaliana* roots resulted in a rapid dose-dependent (from 0.01 to 1 mM) accumulation of intracellular ROS throughout the plasma membrane NOX enzyme. In support of our data, Clark and Roux (2009) pointed out that $O_2^{\bullet -}$ and NO appear to be early signaling intermediates. The present results are going in the same direction and support this role for $O_2^{\bullet -}$ and NO. Reichler et al. (2009) also reported the connection between eATP and NO signaling pathways in the regulation of pollen germination and pollen tube growth. More recently, Weerasinghe et al. (2009) proposed a role of G-proteins in regulating mechano-sensitive ATP release during root growth. Coincidently, eATP and NO act as signaling agents in both plant and animal cells by eliciting a cascade of similar events, including heterotrimeric G protein, H_2O_2 and Ca^{2+} (Besson-Bard et al., 2008; Wu et al., 2008; Li et al., 2009). On the other hand, the redox system through apoplast, membrane and cytoplasm triggers a Ca^{2+} gradient and also involves ROS and G-protein (Joo et al., 2005).

Our current knowledge of redox control predicts that the plant membrane is an important site for perception and transduction of environmental stimuli through redox signaling. Extracellular redox changes may facilitate interactions between receptor proteins containing thiol groups that are sited near the membrane (Foyer and Noctor, 2005). Demidchik et al. (2009) reported that the plasma membrane could be the site where the receptors and several modulators of the eATP signaling pathway converge.

Another key question is how eATP–NO, redox system and growth regulators are interconnected to lead the hypocotyl growth. At least five classes of plant hormones are involved in the process of hypocotyl growth in darkness auxin and brassinosteroid strongly being implied (Vandenbussche et al., 2005). In the process of adventitious rooting in cucumber the relationship between auxin and NO was described (Pagnussat et al., 2003). Then, it would be possible that eATP, NO and auxin may coordinately participate in the elongation of etiolated *A. thaliana* hypocotyls.

On the other hand, the Arabidopsis Brassinosteroid Receptor (AtBRI1) contains a domain that functions as a guanylyl cyclase (GC; Kwezi et al., 2007). GCs catalyze the formation of the second messenger guanosine 3′,5′-cyclic monophosphate (cGMP). Likewise, cGMP has been implicated in an increasing number of plant developmental processes, including responses regulated by auxin and NO (Pagnussat et al., 2003). Our findings indicate that the molecular interaction between eATP–NO, redox system and phytohormones might be more complex than previously thought and might be tissue or response-type dependent.

Acknowledgments

This work was supported by grants from CONICET, Universidad Nacional de Mar del Plata and ANPCyT. L.L. and C.C. are members of CONICET. M.C.T. is a recipient of the post-doctoral fellowship of the same Institution. C.T. is a technical assistant from CIC. We thank Dr. Ana M. Laxalt and Dr. Diego Fiol for their critical readings of the manuscript. We thank the undergraduate students of Plant Physiology course at UNMDP, specially Vanesa Tossi, for inspiring us with comments on this subject.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jplph.2009.11.002.

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