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New insights into NO generation and *AOX1* upregulation in *Chlamydomonas*

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Summary

Emerging evidence indicates a close connection between components mitochondrial electron transport chain (mETC), nitric oxide (NO) and alternative oxidase (AOX) activity in plants. In unicellular algae, AOXs are monomeric fungi-type proteins. We previously showed that in *Chlamydomonas reinhardtii* stress-induced *AOX1* expression was significantly higher under dark conditions. Here we found that in dark aerobic *Chlamydomonas* cells, complex III of mETC produces NO from nitrite, and monomeric AOX1 appeared to be involved in reducing this activity. Moreover, in dark-incubated cells, NO generation is not mediated by nitrate reductase and nitric oxide-forming nitrite reductase complex. Under conditions limiting the flow of electrons through complex III, the expression of *AOX1* but not the *AOX2* expression is dependent on changes in NO levels. Taken together, the data indicate that in the absence of light, NO generated by mETC, is a key molecule controlling *AOX1* levels in *Chlamydomonas* aerobic cells.

Key words: nitric oxide, *Chlamydomonas reinhardtii*; alternative oxidase, nitrate reductase, mitochondrial electron transport chain

Introduction

The mitochondrial electron transport chain (mETC) in some protists, many fungi and plants consists of the ATP-coupling cytochrome (cyt) pathway (complex III, cyt c, cyt oxidase), and the alternative oxidase (AOX) pathway (Finnegan et al., 2004; Millar et al., 2011). As AOX bypasses proton pumping complexes III and IV of the cyt pathway, it reduces ATP generation and the energy yield of respiration (Affourtit et al., 2001).

AOX is a membrane-bound ubiquinol oxidase that directly couples ubiquinol oxidation with O_2 reduction to water (Vanlerberghe and Ordog, 2002; Finnegan et al., 2004). Two types of AOX proteins, homodimeric plant-type and monomeric fungi-type, were identified (Affourtit et al., 2001). In the model alga *Chlamydomonas reinhardtii* (*Chlamydomonas* in the following), AOXs are monomeric fungi-type proteins that are encoded by two genes of discrete subfamilies, *AOX1* and *AOX2* (Dinant et al., 2001).

Nitric oxide (NO) triggers the upregulation of AOX1 in *Chlamydomonas* (Zalutskaya et al., 2017). Although *Chlamydomonas* cells form NO associated with various physiological processes (Hemschemeier et al., 2013; Wei et al., 2014; Minaeva et al., 2017),

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only one enzymatic system producing NO, nitrate reductase – nitric oxide-forming nitrite reductase, was characterized in the alga (Chamizo-Ampudia et al., 2016). Notably, no gene encoding functional NOS (nitric oxide synthase) was identified in *Chlamydomonas* genome so far (Fröhlich et al., 2011; Jeandroz et al., 2016). Furthermore, despite the known contribution of mitochondria to NO production in animal and plant cells (Kozlov et al., 1999; Planchet et al., 2005; Gupta et al., 2018), the participation of mETC in NO generation is currently not fully understood in *Chlamydomonas*. Only complex IV was shown as a potential site for NO generation in this alga (Maxence et al., 2017).

Here we found a new source for a NO generation in *Chlamydomonas*, complex III of mETC. Furthermore, in dark aerobic cells, NO controls the *AOX1* expression and monomeric AOX conversely regulates NO generation by the mETC. This study thus suggests a molecular strategy in algae to coordinate NO and monomeric AOX levels in mitochondria under normoxia conditions.

Material and methods

Strains, growth conditions and chemical treatment

Chlamydomonas strains 21gr *mt*$^+$ (wild type) and 305 (*mt*$^-$*nia1*) were kindly provided by Dr. Emilio Fernandez (University of Cyrdoba, Spain). Cells were grown mixotrophically in tris-acetate-phosphate (TAP) medium (http://www.chlamy.org/TAP.html) under continuous illumination with white light (fluence rate of 45 µmol m^{-2} s^{-1}) at 22 °C with constant orbital agitation at 90 rpm. Cells were collected at the midexponential phase of growth by centrifugation (4000*g*, 5 min), washed twice with nitrogen-free TAP, incubated in the indicated medium and then transferred into darkness. Dissolved oxygen was assayed using a Clark-type electrode to ensure that aerobic conditions were maintained.

Chlorophyll content was determined using ethanol extraction as described (Minaeva et al., 2017). When indicated antimycin A, myxothiazol or salicylhydroxamic acid (SHAM) were added to the medium to a final concentration of 100 µM, 6 µM and 2mM, respectively. All chemical compounds are from Sigma-Aldrich.

Measurement of NO

Chlamydomonas cells (45 µg/ml chlorophyll) were incubated in the presence of 1 µM (4-amino-5-methylamino-2’7’-difluorofluorescein diacetate) dye (DAF-FM DA, Sigma-Aldrich). After 15 min the cells were washed, resuspended in indicated medium and then incubated for an additional 30 min to allow complete de-esterification of the intracellular diacetates. Intracellular production of NO was measured using a microplate reader CLARIOstar (BMG). Excitation and emission wavelengths were set at 483±14 and 530±30 nm, respectively. Fluorescence intensity was expressed as arbitrary units per µg chlorophyll. Cell autofluorescence was subtracted from the total fluorescence obtained. Three technical replicates per condition were included on each plate and each experiment was performed at least three times independently.

Gene expression analysis

RNA was isolated as described previously (Ermilova et al., 2010). The quality of the RNA preparations was estimated by agarose gel electrophoresis, and RNA concentration and purity were determined spectrophotometrically (SmartSpec Plus, Bio-Rad). Reverse transcription was performed with RevertAid H MinusFirst Strand cDNA Synthesis Kit according to the manufacturerґs instructions (Thermo Scientific). The primer pairs used for real time qPCR were 5′-ACTTGGATCTGTGTGCCTGG-3′ and 5′-CTGGGCCGGTATCTACGTTC-3′ for *AOX1*, 5′-GAGTTTCAGGACCCGGATGG-3′ and 5′-AATAAACCGCAACCCCAGGT-3′ for *AOX2*, and 5′-CTTCTCGCCCATGACCAC-3′ and 5′-CCCACCAGGTTGTTCTTCAG-3′ for *RACK1*. Real time qPCR was performed on the Light Cycler Instrument (CFX96 Real-Time PCR Detection System, Bio Rad) using SYBR Green I as described (Zalutskaya et al., 2016). Gene expression ratios were normalized to *RACK1* (receptor of activated protein kinase C; Cre13.g599400) using the ΔΔCt method (Livak et al., 2001). Antimycin A, myxothiazol or SHAM treatments had no effect on the accumulation of *RACK1* transcripts in *Chlamydomonas* cells. The accuracy and reproducibility of the real time assay was determined from low variation in C_T values across replicates. Values were obtained from at least three biological replicates; each replicate

was analyzed three times. Student's t-tests were used for statistical comparisons. P-values of <0.05 were considered as significant.

Results and discussion

Dark-induced NO generation is not dependent on NR

Our previous studies showed that stress-induced *AOX1* expression was significantly higher under dark conditions (Zalutskaya et al., 2015, 2016, 2018). How can dark treatment lead to increased *AOX1* induction under various stress conditions? Because NO has been implicated in modulating *AOX1* expression and activity (Zalutskaya et al., 2017), we explored the potential NO generation under dark treatment further. The levels of NO were first verified in WT (21gr) strain transferred to the dark (Fig. 1A). In response to the dark treatment in ammonium-containing medium, NO amount was increased to only 1.5-fold after 1 h of incubation. When *Chlamydomonas* cells were exposed to nitrate, we observed the higher NO amounts than in ammonium medium. However, the highest levels of NO generation were reached when cells were incubated in 10 mM nitrite (Fig. 1A). Therefore, we chose 10 mM nitrite-containing medium for further experiments. Importantly, in all experiments no significant drop in the dissolved oxygen levels was monitored, showing only slight decrease (about 7-12%) after 3 h of incubation in the dark.

In higher plants, the crucial role of NR in NO production in various processes has been demonstrated (Desikanet al., 2002; Hao et al., 2010; Kolbert et al., 2010; Xie et al., 2013; Lombardo et al., 2012; Santolini et al., 2016). In *Chlamydomonas*, it has been shown that NR can interact with the partner protein nitric oxide-forming nitrite reductase (NOFNiR) to catalyze the formation of NO from nitrite (Chamizo-Ampudia et al., 2016). Moreover, our findings suggest that NR-NOFNiR system plays a positive role in *THB1* (truncated hemoglobin 1) transcription via generation of NO from nitrite in S-starved cells in the light (Zalutskaya et al., 2018). We further asked whether NR-deficient aerobic cells could generate NO in the dark. We used *nit1* mutant, 305 (Sosa et al., 1978). During dark incubation, the formation of NO was clearly detected in NR-defective mutant strain (Fig. 1B), indicating that NR is not essential to generate NO in dark cells under aerobic conditions. Moreover, NO levels was significantly higher in nit1 strain than in WT (compare Fig. 1 A and B), hinting that NO quenching could be impaired in this strains. These results prompted us to explore the source of NO formation in dark *Chlamydomonas* cells under normoxia.

Complex III is a source for NO generation in aerobic dark-incubated cells

The mitochondrial electron transport chain (mETC) has been shown to produce NO in plant cells under hypoxia (Tischner et al., 2004; Planchet et al., 2005). It was shown that *Chlamydomonas* under normoxic conditions can reduce nitrite into NO using a mitochondrial cytochrome c oxidase (Maxence et al., 2017). Interestingly, in animal mitochondria, complex III can be another source for NO generation from nitrite under normoxia (Nohl et al., 1999). In addition, in tobacco leaves, complex III is required for NO production under aerobic conditions (Alber et al., 2017). We further asked whether complex III could be a site of NO formation in aerobic dark-incubated *Chlamydomonas* cells. We used previously described inhibitors of the complex III, myxothiazol (Qo-site inhibitor) and antimycin A (Qi -site inhibitor) (Planchet et al., 2005; Gupta et al., 2005). To our data, NO levels were strongly increased in antimycin A-treated WT cells (Fig. 2A). The second inhibitor, myxothiazol, also increased NO generation. However, NO amounts after myxothiazol treatment were significantly lower than after antimycin A treatment. Notably, these results are well consistent with data of Alber et al. (2017) obtained in tobacco leaves. It was detected that electron pressure in the Q-cycle of complex III resulted in single electron leak to nitrite generating NO. We found that myxothiazol reduced the antimycin A-modulated increase in NO levels (Fig. 2A), suggesting that the Q-cycle of complex III can generate NO from nitrite in dark *Chlamydomonas* cells under normoxia.

NO generated via complex III is a retrograde signal controlling *AOX1* expression

As expression of AOX1 in *Chlamydomonas* was reported to be increased by antimycin A (Molen et al., 2006), we asked whether or not the amounts of *AOX1* transcripts correlated with NO levels in cells treated with inhibitors of complex III. Indeed, the

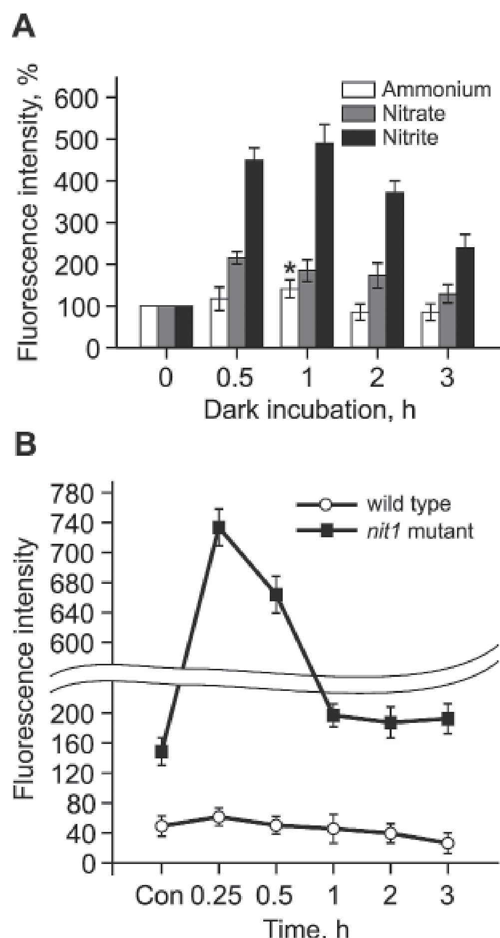

Fig. 1. The effects of dark incubation on NO production by *Chlamydomonas* strains under normoxia. A – NO levels were determined in the cells of 21gr strain (wild type) grown in ammonium-containing medium in the light and transferred into the dark to media containing 7 mM ammonium, 7 mM nitrate or 10 mM nitrite. Fluorescence intensity due to intracellular NO was determined using 1 µM DAF-FM DA and was expressed as arbitrary units per µg chlorophyll. Cell autofluorescence was subtracted from the total fluorescence obtained. Fluorescence in the cells grown in the light in ammonium-containing medium is considered as control (set to 100%). Data are the means±SE (error bars) from three independent experiments. *Asterisk* denotes statistical significance of means compared with the control at the 95% level. B – NO levels were determined in cells of wild type (21gr) and nitrate reductase deficient strain (305) grown in ammonium-containing medium in the light and transferred into the dark to 10 mM nitrite-containing medium. Following 0.5 h pre-incubation in the dark (Con), the cells were used for analysis of NO generation. Fluorescence intensity due to intracellular NO was determined using 1 µM DAF-FM DA and was expressed as arbitrary units per µg chlorophyll. Data are the means±SE (error bars) from three independent experiments.

AOX1 transcript had a 3.8- and 3.2-fold increase in the antimycin A-treated cells after 1h and 2h of incubation compared with the untreated cells (Fig. 2B). Similarly, but to a less extent, *AOX1* expression relative to that in the control cells was elevated in cells after myxothiazol treatment. Interestingly, the increase of *AOX2* expression in cells treated with antimycin A or myxothiazol was not observed (Fig. 2B, insertion). Thus, the complex III-dependent NO generation appeared to be involved in the signal regulation of *AOX1* transcription but not of *AOX2* transcription.

AOX1 IS A FACTOR CONTROLLING NO GENERATION BY THE mETC

Scavenging of NO is essential for cells as excess of NO can react rapidly with other toxic free radicals and proteins. Because homodimeric AOX can regulate mitochondrial NO production in higher plants (Cvetkovska and Vanlerberghe, 2012; Cvetkovska et al., 2014), we investigated the possibility that monomeric AOX can also prevent NO formation in dark-adapted algal cells under normoxic conditions. To identify the potential role of AOX in the control of NO amounts, we analyzed salicylhydroxamic acid (SHAM) that is inhibitor of the alternative electron transport pathway. Interestingly, SHAM application led to enhanced NO production from nitrite and NO generation became even stronger when SHAM was used in addition to antimycin A (Fig. 2C), suggesting that monomeric AOX can scavenge mitochondrial NO.

Next, we asked the question whether the increased levels in NO generation in *nit1* mutant was accompanied by changes in the *AOX1* expression. In WT, the transcript level increased rapidly to approximately 4.5–4-fold that of the control after 30 min and 1 h, respectively .The dark incubation of *nit1* cells in nitrite-containing medium resulted in only about 2-fold induction of this gene over the 2-h exposure period (Fig. 2D), hinting that AOX-dependent scavenging mechanism for NO could be less effective in this strain that in the WT. In further considering the interactions between AOX1 and NO in *Chlamydomonas*, it is clear that this needs to be further characterised in the AOX1-unerexpressing strains.

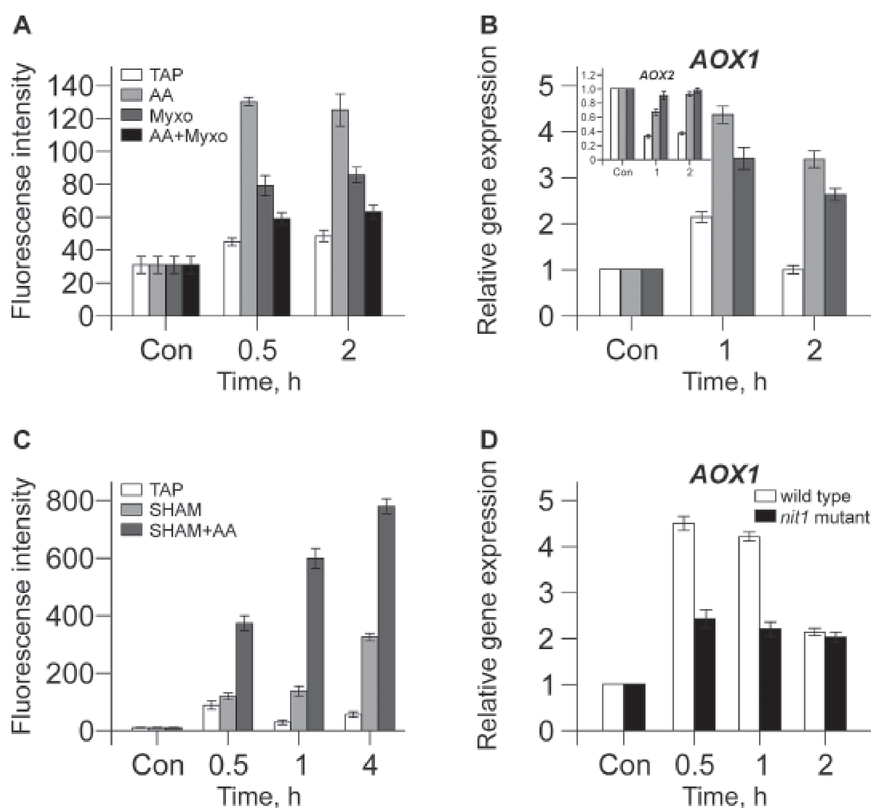

Fig. 2. Changes in NO levels and *AOX1* expression in *Chlamydomonas* strains in response to treatments with complex III and AOX inhibitors under normoxia. A – Effects of complex III inhibitors on NO generation in WT. Cells were grown and treated as described in Fig. 1B. Following 0.5 h pre-incubation in the dark (Con), antimycin A (AA, 100 µM), myxothiazol (Myxo, 6 µM) or antimycin A + myxothiazol were added to the medium. Fluorescence intensity due to intracellular NO was determined using 1 µM DAF-FM DA and was expressed as arbitrary units per µg chlorophyll. Data are the means±SE (error bars) from four independent experiments. B – Effects of complex III inhibitors on *AOX1* and *AOX2* expression in WT. Cells were treated as described in (A). Relative expression levels were normalized with the gene expression of *RACK1* and calculated using ΔCT. Values are means ± SE of three biological replicates and three technical replicates. C – Effects of AOX inhibitor SHAM on NO generation in WT. Cells were grown and treated as described in (A). Following 0.5 h pre-incubation in the dark (Con), SHAM (2 mM) or SHAM + antimycin A (100 µM) were added to the medium. Fluorescence intensity due to intracellular NO was determined using 1 µM DAF-FM DA and was expressed as arbitrary units per µg chlorophyll. Data are the means±SE (error bars) from three independent experiments. D – Time course of the *AOX1* transcripts accumulation during dark incubation of WT and *nit1* cells in nitrite-containing medium. Cells were grown in ammonium-containing medium in the light (Con) and transferred into the dark to 10 mM nitrite-containing medium. Relative expression levels were normalized with the gene expression of *RACK1* and calculated using ΔCT. Values are means ± SE of three biological replicates and three technical replicates.

Conclusions

Current understanding concerning the regulatory mechanisms of monomeric alternative oxidases in organisms is limited. The results provide substantial evidence that complex III of mETC produces NO from nitrite in aerobic dark-adapted *Chlamydomonas*, and monomeric AOX1 appeared to be involved in reducing this activity. Under conditions limiting the flow of electrons through complex III, *AOX1* expression is dependent on changes in NO levels, indicating that NO is a key molecule controlling AOX1 amounts in dark *Chlamydomonas* cells under normoxia. To our knowledge, we present the first investigations on the effects of NO generated by mETC on expression of a monomeric type of alternative oxidase.

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References

Affourtit C., Albury M.S., Crichton P.G. and Moore A.L. 2001. Exploring the molecular nature of alternative oxidase regulation and catalysis. FEBS Lett. 510, 121–126.

Alber N.A., Sivansan H. and Vanlerberghe G.C. 2017. The occurrence and control of nitric oxide generation by the plant mitochondrial electron transport chain. Plant Cell Environ. 40, 1074–1085.

Chamizo-Ampudia A., Sanz-Luque E., Llamas A., Ocana-Calahorro F., Mariscal V., Carreras A., Barroso J.B., Galvan A. and Fernandez E. 2016. A dual system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO production in *Chlamydomonas.* Plant Cell Environ. 39, 2097–2107.

Cvetkovska M., Dahal K., Alber N.A., Jin C., Cheun M. and Vanlerberghe G.C. 2014. Knockdown of mitochondrial alternative oxidase induces the 'stress state' of signaling molecule pools in *Nicotiana tabacum*, with implications for stomatal function. New Phytologist. 203, 449–461.

Cvetkovska M. and Vanlerberghe G.C. 2012. Alternative oxidase modulates leaf mitochondrial concentrations of superoxide and nitric oxide. New Phytologist. 195, 32–39.

Desikan R., Griffiths R., Hancock J. and Neill S. 2002. A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. U.S.A. 99, 16314–16318.

Dinant M., Baurain D., Coosemans N., Joris B. and Matagne R.F. 2001. Characterization of two genes encoding the mitochondrial alternative oxidase in *Chlamydomonas reinhardtii.* Curr. Genet. 39, 101–108.

Ermilova E., Zalutskaya Z., Nikitin M., Lapina T. and Fernández E. 2010. Regulation by light of ammonium transportsystems in *Chlamydomonas reinhardtii.* Plant Cell Environ. 33, 1049–1056.

Finnegan P.M., Soole K.L. and Umbach, A.L. 2004. Alternative mitochondrial electron transport proteins in higher plant. In: Advances in Photosynthesis and Respiration, vol. 17, Plant Mitochondria: From Genome to Function (Eds: Day D.A., Millar A.H and Whelan J.). Kluwer Academic Press, The Netherlands, pp. 163–230.

Fröhlich A. and Durner J. 2011. The hunt for plant nitric oxide synthase (NOS): is one really needed?. Plant Science. 181, 401–404.

Gupta K.J., Kumari A., Florez-Sarasa I., Fernie A.R. and Igamberdiev A.U. 2018. Interaction of nitric oxide with the components of the plant mitochondrial electron transport chain. J. Exp. Bot. 69, 3413–3424.

Gupta K.J., Stoimenov M. and Kaiser W.M. 2005. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, *in vitro* and *in situ*. J. Exp. Bot. 56, 2601–2609.

Hao F., Zhao S., Dong H., Zhang H., Sun L. and Miao C. 2010. Nia1 and Nia2 are involved in exogenous salicylic acid-induced nitric oxide generation and stomatal closure in *Arabidopsis.* J. Integr. Plant Biol. 52, 298–307.

Hemschemeier A., Duner M., Casero D., Merchant S.S., Winkler M. and Happe T. 2013. Hypoxic survival requires a 2-on-2 hemoglobin in a process involving nitric oxide. Proc. Natl. Acad. Sci. U.S.A. 110, 10854–10859.

Jeandroz S., Wipf D., Stuehr D.J., Lamattina L., Melkonian M., Tian Z., Zhu Y., Carpenter E.J., Wong G.K.S. and Wendehenne D. 2016. Occurrence, structure, and evolution of nitric oxide synthase–like proteins in the plant kingdom. Sci. Signal. 9, 417.

Kolbert Z., Ortega L. and Erdei L. 2010. Involvement of nitrate reductase (NR) in osmotic stress-induced NO generation of *Arabidopsis thaliana* L. roots. J. Plant Physiol. 167, 77–80.

Kozlov A.V., Staniek K. and Nohl H. 1999. Nitrite reductase activity is a novel function of mammalian mitochondria. FEBS Lett. 454, 127–130.

Livak K.J. and Schmittgen T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔCT method. Methods. 25, 402–408.

Lombardo M.C. and Lamattina L. 2012. Nitric oxide is essential for vesicle formation and trafficking in *Arabidopsis* root hair growth. J. Exper. Bot. 63, 4875–4885.

Maxence P., Wheeler D., Shilton A., Packer M.A., McLenachan P.A., Sanz-Luque E., Ocana-Calahorro F., Fernandez E. and Guieysse B. 2017. The biosynthesis of nitrous oxide in the green alga *Chlamydomonas reinhardtii.* Plant J. 91, 45–56.

Millar A.H., Whelan J., Soole K.L. and Day D.A. 2011. Organization and regulation of mitochondrial respiration in plants. Annu. Rev. Plant Biol. 62, 79–104.

Minaeva E., Zalutskaya Z., Filina V. and Ermilova E. 2017. Truncated hemoglobin 1 is a new player in *Chlamydomonas reinhardtii* acclimation to sulfur deprivation. PLoS One. 12 (10), e0186851.

Molen T.A., Rosso D., Piercy S. and Maxwell D.P. 2006. Characterization of the alternative oxidase of *Chlamydomonas reinhardtii* in response to oxidative stress and a shift in nitrogen source. Physiol. Plant. 127, 74–86.

Nohl H., Staniek K. and Kozlov A.V. 1999. Involvement of mammalian mitochondria in recycling of the NO-metabolite nitrite to nitric monoxide. Free Radical Biol. Med. 27, S82–S82.

Planchet E., Gupta K.J., Sonoda M. and Kaiser W.M. 2005. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J. 41, 732–743.

Santolini J., Andrй F., Jeandroz S. and Wendehenne D. 2016. Nitric oxide synthase in plants: Where do we stand?. Nitric Oxide. 63, 30–38.

Sosa F.M., Ortega T. and Barea J.L. 1978. Mutants from *Chlamydomonas reinhardii* affected in their nitrate assimilation capability. Plant Science Lett. 11, 51–58.

Tischner R., Planchet E. and Kaiser W.M. 2004. Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. FEBS Lett. 576 (1–2), 151–155.

Vanlerberghe G.C. and Ordog S.H. 2002. Alternative oxidase: integrating carbon metabolism and electron transport in plant respiration. In: Advances in Photosynthesis and Respiration, vol. 12, Photosynthetic Nitrogen Assimilation and Associated Carbon and Respiratory Metabolism (Eds: Foyer C.H. and Noctor G.). Kluwer Academic Publishers, The Netherlands, pp. 173–191.

Wei L., Derrien B., Gautier A., Houille-Vernes L., Boulouis A., Saint-Marcoux D., Malnoë A., Rappaport F., de Vitry C., Vallon O., Choquet Y. and Wollman F.-A. 2014. Nitric oxide-triggered remodeling of chloroplast bioenergetics and thylakoid proteins upon nitrogen starvation in *Chlamydomonas reinhardtii*. Plant Cell. 26, 353–372.

Xie Y., Mao Y., Lai D., Zhang W., Zheng T. and Shen W. 2013. Roles of NIA/NR/NOA1-dependent nitric oxide production and HY1 expression in the modulation of *Arabidopsis* salt tolerance. J. Exper. Bot. 64, 3045–3060.

Zalutskaya Z., Filina V., Ostroukhova M. and Ermilova E. 2018. Regulation of alternative oxidase 1 in *Chlamydomonas reinhardtii* during sulfur starvation. Eur. J. Protistol. 63, 26–33.

Zalutskaya Z., Lapina T. and Ermilova E. 2015. The *Chlamydomonas reinhardtii* alternative oxidase 1 is regulated by heat stress. Plant Physiol. Biochem. 97, 229–234.

Zalutskaya Z., Ostroukhova M. and Ermilova E. 2016. The *Chlamydomonas* alternative oxidase 1 is regulated by cadmium stress: new insights into control of expression. Environ. Exp. Bot. 130, 133–140.

Zalutskaya Z., Ostroukhova M., Filina V. and Ermilova E. 2017. Nitric oxide upregulates expression of alternative oxidase 1 in *Chlamydomonas reinhardtii*. J. Plant Physiol. 219, 123–127.

Zalutskaya Z., Filina V. and Ermilova E. 2018. Sulfur deprivation-induced expression of THB1, a *Chlamydomonas reinhardtii* truncated hemoglobin, is mediated by nitrate reductase-dependent NO production. Protistology. 12, 97–108.

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