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Nitric oxide and truncated hemoglobin 1 in regulation of sulfur deprivation responses in *Chlamydomonas*

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Statement of the Problem: Sulfur (S) is an essential element among catalysts and intermediates of primary metabolism. S can be limiting in the environment and strongly influence ecosystem composition. During S deprivation, metabolism of *Chlamydomonas* cells is refocused on both scavenging the nutrient and remodeling primary metabolism. Although some signaling proteins and regulators of S-specific responses have been identified, the mechanisms triggering the coordinated responses in different cellular compartments are not absolutely clear. Previously, we have reported that nitric oxide is generated upon S deprivation.

Purpose: The purpose of this study is to elucidate the role of nitric oxide (NO) and truncated hemoglobin 1 (THB1) in modulating early responses to S deficiency in different compartment of *Chlamydomonas* cells.

Methodology & Theoretical Orientation: To examine transcriptional regulation of a subset of S limitation-responsive genes and role of NO and THB1 in signaling pathway associated with S deprivation, real-time PCR analysis and artificial microRNA method were employed. A comparative analysis of gene expression and NO generation in wild type, nitrate reductase mutants and THB1 knock-down transformant was utilized to understand the functional consequences of NO production.

Findings: In S-free medium, *Chlamydomonas* cells produced NO apparently via nitrate reductase. We found that in S-limited cells NO is important to upregulate some S deficiency-inducible genes (THB1) and repress the others (FDX5, ARS1, ARS2, SULTR2, SLT1 and SLT2). THB1 is involved in this NO-dependent process.

Conclusion & Significance: Together, the results demonstrated that THB1 has implicated to function as NO regulator (via conversion of NO into nitrate) and thereby initiate NO-based signaling cascades in S-depleted cells. Moreover, NO generation may be regarded as an early trigger, which contributes to *Chlamydomonas* adaptability to S starvation.

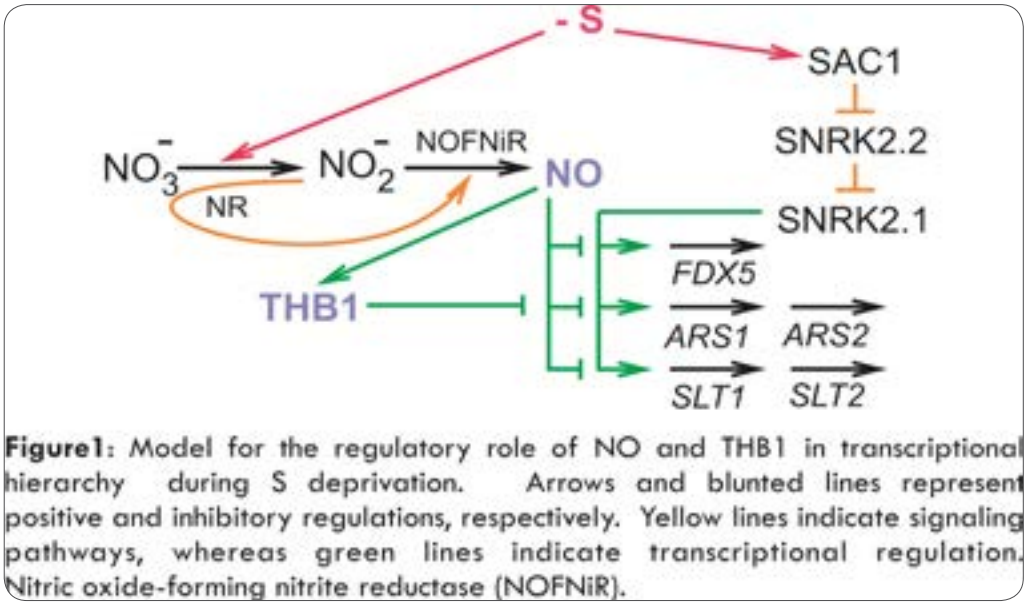

Figure1: Model for the regulatory role of NO and THB1 in transcriptional hierarchy during S deprivation. Arrows and blunted lines represent positive and inhibitory regulations, respectively. Yellow lines indicate signaling pathways, whereas green lines indicate transcriptional regulation. Nitric oxide-forming nitrite reductase (NOFNiR).

Recent Publications

1. Aksoy M, Pootakham W, Pollock S V, Moseley J L, Gonzalez-Ballester D and Grossman A R (2013) Tiered regulation of sulfur deprivation responses in *Chlamydomonas reinhardtii* and identification of an associated regulatory factor. Plant Physiology 162:195-211.
2. 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.
3. Zalutskaya Z, Minaeva E, Filina V, Ostroukhova M and Ermilova E (2018) Regulation of sulfur deprivation-induced expression of the ferredoxin-encoding FDX5 gene *Chlamydomonas reinhardtii* in aerobic conditions. Plant Physiology and Biochemistry 123:18-23.
4. Zalutskaya Z, Filina V, Ostroukhova M and Ermilova E (2018) Regulation of alternative oxidase 1 in *Chlamydomonas reinhardtii* during sulfur starvation. European Journal of Protistology 63:26–33.
5. Zhang Z, Shrager J, Jain M, Chang C W, Vallon O and Grossman A R (2004) Insights into the survival of *Chlamydomonas reinhardtii* during sulfur starvation based on microarray analysis of gene expression. Eukaryotic Cell 3(5):1331-1348.

Biography

Elena Ermilova is a full Professor and Head of Laboratory at Saint-Petersburg State University. She has her expertise in nitrogen metabolism and PII signal transduction in green and red algae, and land plants. She has extensively studied the unicellular green algae *Chlamydomonas reinhardtii* and identified new functions associated with stress acclimation. She also studies the regulation of sulfur metabolism.

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