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Independent evolution of the thioredoxin system with diverse phylogenetic origins in photosynthetic *Paulinella* species

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Abstract

Redox regulation in phytoplankton is critical to monitor and stabilize metabolic pathways under changing environmental conditions[1]. In plastids, the thioredoxin (TRX) system is linked to photosynthetic electron transport and fine tuning of metabolic pathways to fluctuating light levels. Expansion of the number of redox signal transmitters and their protein targets, as seen in plants, is believed to increase cell robustness[2]. In this study, we searched for genes related to redox regulation in the genome of the photosynthetic amoeba *Paulinella micropora* KR01 (hereafter, KR01). The genus *Paulinella* includes testate filose amoebae, in which a single clade acquired a photosynthetic organelle, the chromatophore, from an alpha cyanobacterial donor[3]. This independent primary endosymbiosis occurred relatively recently (~ 124 Ma), when compared to Archaeplastida (> 1 Ga), making photosynthetic *Paulinella* a valuable model for studying the earlier stages of primary endosymbiosis[4]. Our comparative analysis demonstrates that this lineage has evolved a thioredoxin system similar to other algae, relying however on genes with diverse phylogenetic origins (i.e., the endosymbiont, host, bacteria, red algae). One TRX of eukaryotic provenance is targeted to the chromatophore, implicating host-endosymbiont coordination of redox regulation. A chromatophore targeted glucose-6-phosphate dehydrogenase of red algal origin suggests that *Paulinella* exploited the existing redox regulation system in Archaeplastida to foster integration. Our study elucidates the independent evolution of the thioredoxin system in photosynthetic *Paulinella*, whose parts derive from the existing genetic toolkit in diverse organisms.

eTOC blurb

In plastids, the thioredoxin system is linked to photosynthetic electron transport and fine tuning the response to fluctuating light levels. Photosynthetic *Paulinella,* the only other known case

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D.L. and H.S.Y. designed the project. D.L. performed bioinformatic analysis. All authors wrote, read and approved the manuscript before submission.

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of primary photosynthetic organelle origin, have a similar system using genes with diverse phylogenetic origins.

TRXs are thiol-disulfide oxidoreductases that participate in the regulation of metabolism and defense against oxidative stress and respond to environmental signals[5]. In the KR01 nuclear genome, there are 9 gene candidates, each of which contains a single TRX domain with a redox-active cysteine pair[1]. Apart from the TRXs encoded in the nucleus, the chromatophore genome encodes two such genes, whose products remain in the chromatophore, based on proteomic data from *P. chromatophora*[6] (Fig. 1). Phylogenetic analysis of TRX genes shows that the nuclear encoded copies are of eukaryotic derivation except for g38191.t1, which groups with bacteria (Data S2A). One (g35100.t1) of the eukaryotic TRX proteins is likely to be chromatophore targeted because it contains a distinctive chromatophore transit peptide (crTP). Proteomic data from *P. chromatophora* demonstrates that the protein derived from the ortholog of this gene (i.e., g35100.t1) is present in the chromatophore of this species[6]. In *Arabidopsis thaliana*, there are ~20 TRXs, all of which are nuclear-encoded, and five types (f, m, x, y, and z) are imported into the plastid after translation. Among these five types, four have originated from cyanobacteria *via* endosymbiotic gene transfer (EGT)[5]. In red algae, one TRX encoding gene is present in the plastid genome that shares highest sequence similarity to type-m TRXs of *A. thaliana*[7]. In the case of photosynthetic *Paulinella,* when compared to *A. thaliana*, the amoeba appears to have jettisoned more of the endosymbiont derived TRX genes, perhaps indicating a lower level of integration between the nucleus and chromatophore. However, it is interesting to note that chromatophore origin precipitated the targeting of an eukaryote derived TRX to the novel organelle, likely reflecting selection pressure to expand the number of redox signal transmitters to respond to environmental cues[2]. Photosynthetic *Paulinella* relies on thioredoxin reductases (TRs) that are encoded in the chromatophore, whereas in green plants, TRs are nuclear encoded (Table S1).

Activity of a key enzyme in the oxidative pentose phosphate pathway (OPPP), plastid-targeted glucose-6-phosphate dehydrogenase (G6PDH), is regulated by light *via* TRX[5]. In the case of KR01, we found three G6PDHs encoded in the nucleus, all of which are of eukaryotic origin (Data S2B). However, two of these genes (g40784.t1, g9229.t1) are most closely related to red algal homologs, implying their origin *via* horizontal gene transfer (HGT). Interestingly, the two HGT-derived genes encode a crTP and contain the same redox-active cysteine pair as plastid encoded G6PDH. Moreover, one of these genes (g40784.t1) exhibits a diurnal expression pattern[8]. This result suggests that photosynthetic *Paulinella* acquired G6PDH genes that had already evolved redox-active cysteine residues during Archaeplastida evolution. Given sequence conservation over most of the protein sequence, it is less likely that convergent evolution targeted only the active sites or structural elements in these enzymes, after their provenance in *Paulinella*. It has been suggested that nuclear-encoded genes acquired from taxa that have previously undergone endosymbiosis may aid novel endosymbiont integration[9]. This is because the expression of these genes is already under host control and they contain import signals that can be more easily reused to target proteins to a new compartment[10]. The foreign genes encoding chromatophore-targeted G6PDH in *Paulinella* may have been acquired *via* phagotrophy involving red algal cells and likely played important roles in redox regulation prior to endosymbiosis.

In addition to G6PDH in plastids, six Calvin-Benson cycle enzymes are light-regulated through the ferredoxin-linked TRX system[5]. In the case of KR01, most of these enzymes are encoded in the chromatophore (organelle) genome, unlike other Archaeplastida (Table S1). Another well studied light-regulated enzyme is chloroplast NADP-dependent malate dehydrogenase[1]. We found four malate dehydrogenase genes (MDH) that are nuclear-encoded, of which, one (g8917.t1) is of bacterial origin and contains a crTP (Data S2C). Proteome data from *P. chromatophora* demonstrates that the protein derived from the ortholog of g8917.t1 in this species is targeted to the chromatophore. In *A. thaliana* and other members of the green lineage, plastid-targeted NADP-MDH is of prokaryote (likely chlamydial) origin. Irrespective of whether chromatophore targeted MDH is redox sensitive or not, this protein apparently plays an important role in primary endosymbiosis because the ancestor of the green lineage and *Paulinella* have acquired this gene from bacteria in two independent HGT events.

Acquisition of the capacity for oxygenic photosynthesis comes with the issue of reactive oxygen species (ROS) production by the electron transport chain (as in mitochondria). In the presence of excessive light energy, electrons may exit the thylakoid membrane and generate ROS. Therefore, it is necessary for algae and plants to sense and regulate such imbalances. The thiol-based redox regulation is a response to this stressor in plastids. When we searched for components of the TRX system that are well characterized in plastids, we found that photosynthetic *Paulinella* species have cobbled together a similar system using genes with diverse phylogenetic origins (i.e., endosymbiont, host, bacteria, red algae). Compared to Archaeplastida, the chromatophore genome still retains many genes related to redox regulation (e.g., TRX, GRX, TRs, see Table S1). Interestingly, chromatophore targeted G6PDH originated from red algae suggesting that *Paulinella* likely “stole” this function that had developed earlier in Archaeplastida. Independent evolution of the thioredoxin system in the chromatophore underlines the importance of this response to redox stress associated with plastid origin. Because our results are based on comparative methods, it is necessary to validate redox sensitivity of gene functions using experimental approaches. Mass spectrometry-based proteomics using cysteine selective isobaric and isotopic tags offers one avenue of investigating redox regulation in this fascinating amoeba lineage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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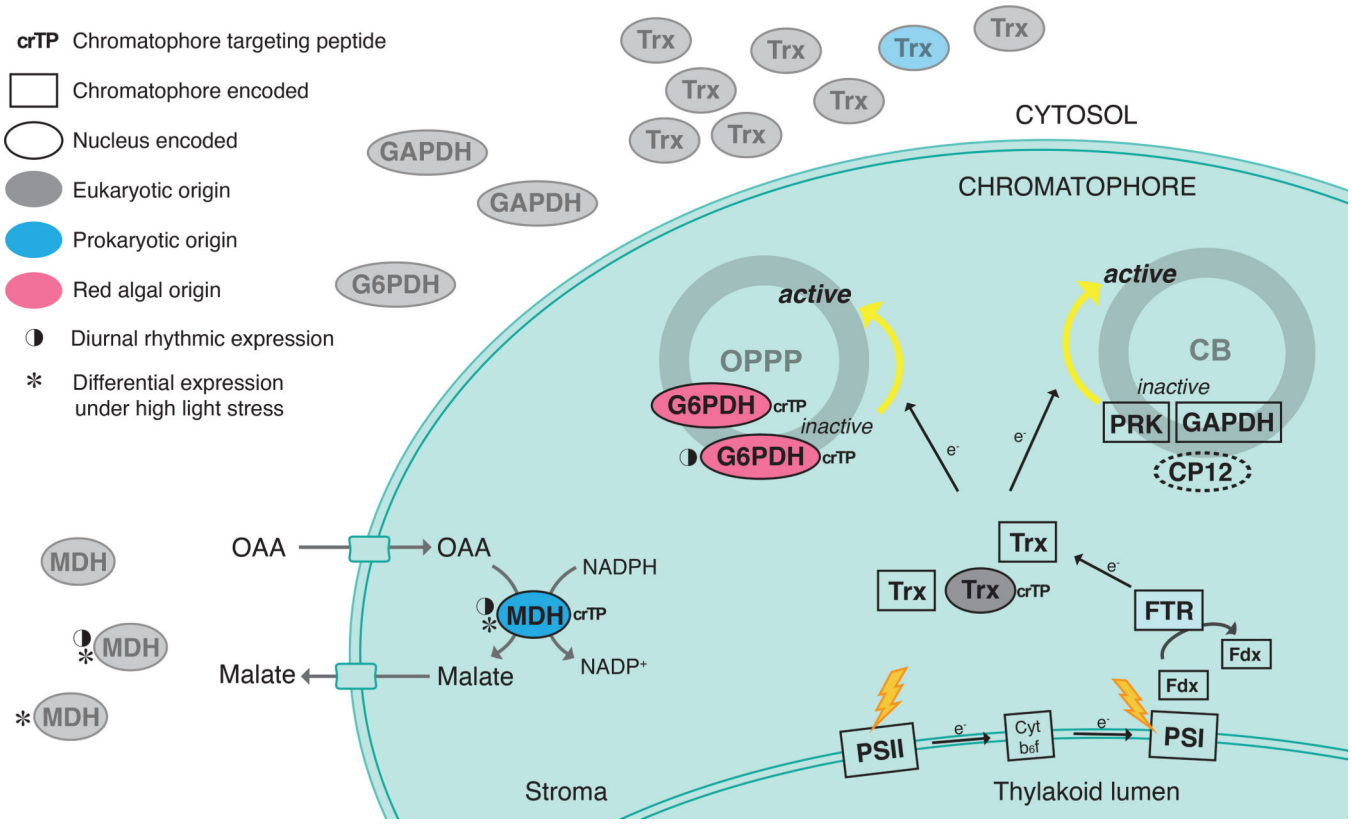

Figure 1.

Putative thioredoxin system in the chromatophore of photosynthetic *Paulinella* species. When the chromatophore is exposed to light (yellow lightning bolt), induced reducing power is transferred from ferredoxin (Fdx) in the electron transport chain to specific target proteins *via* the ferredoxin-thioredoxin reductase (FTR)/thioredoxin (Trx) redox cascade. Thereafter, the reduced thioredoxin acts as a molecular on/off switch for redox regulated proteins by reducing the disulfide bond of enzymes involved in photosynthesis. The genes shown here are discussed in the main text. The localization of proteins to the chromatophore is supported by proteomic analysis of *P. chromatophora*, except for CP12, that did not have an identifiable ortholog in these data. Therefore, the location of this protein is unclear, and indicated with a dashed line.