

Article Addendum

Implication of chlorophyll biosynthesis on chloroplast-to-nucleus retrograde signaling

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The biogenesis and function of chloroplast are controlled both by anterograde mechanisms involving nuclear-encoded proteins targeted to chloroplast and by retrograde signals from plastid to nucleus contributing to regulation of nuclear gene expression. A number of experimental evidences support the implication of chlorophyll biosynthesis intermediates on the retrograde signaling, albeit an earlier-postulated direct link between accumulation of chlorophyll intermediates and changes in nuclear gene expression has recently been challenged. By characterization of *Arabidopsis* mutants lacking the chloroplast localized NADPH-thioredoxin reductase (NTRC) we have recently proposed that imbalanced activity of chlorophyll biosynthesis in developing cells modifies the chloroplast signals leading to alterations in nuclear gene expression. These signals appear to initiate from temporal perturbations in the flux through the pathway from protoporphyrin to protochlorophyllide rather than from the accumulation of a single intermediate of the tetrapyrrole pathway.

Orchestrated regulation of gene expression in the nucleus and plastids is crucial for the proper biogenesis of the organelle during the development and for the acclimation of plants to environmental cues. Multiple potential candidates for initiating plastidial signals have been recognized, including intermediates of the tetrapyrrole biosynthetic pathway, redox state of chloroplast electron transfer components and reactive oxygen species (ROS). These multiple signaling pathways are likely to interact with each others, resulting in a complex signaling network between plastid and nucleus (reviewed in ref. 1).

Control of Nuclear Gene Expression by the Intermediates of Plastidial Tetrapyrrole Pathway

The tetrapyrrole biosynthetic pathway resides in plastids and produces a number of important cofactors for cellular apoproteins, including chlorophylls, heme and the chromophore for the red light photoreceptors.^{2,3} The pathway is regulated on multiple levels in cells to ensure coordinated productions of ligands and apoproteins and to avoid accumulation of harmful phototoxic intermediates of the pathway. Both the mutations in the genes of tetrapyrrole biosynthesis and the treatment with herbicide Norflurazon have evidenced that the intermediates of tetrapyrrole pathway are involved in retrograde signaling from chloroplast to nucleus.⁴⁻⁸ Photobleaching of chloroplasts by Norflurazon has shown to repress concomitantly the expression of photosynthetic genes in the nucleus.^{6,7} Norflurazon treatments were used to screen *Arabidopsis* mutants with defects in retrograde signaling pathway, which resulted in the isolation of genome uncoupled (GUN) mutants. In four out of five *gun* mutants, the mutated genes encode the tetrapyrrole biosynthetic enzymes or their regulatory components (Fig. 1, reviewed in ref. 7). Characterization of the *gun* mutants led to the construction of a model of plastid-to-nucleus retrograde signaling, in which the accumulation of Mg-protoporphyrin IX (MgProto) in plastids initiates the signal repressing the expression of nuclear photosynthetic genes.^{1,6,7} MgProto has also been reported to exit plastid and translocate to cytoplasm⁹ and the AP2-type transcription factor ABI4 has been shown to act downstream of MgProto in nucleus.¹⁰

Recently this model has been challenged by extended analyses of tetrapyrrole intermediates in wild type and *gun* mutants treated with and without Norflurazon under various growth conditions.^{11,12} In these studies, no correlation between the expression of photosynthetic nuclear genes and the accumulation of tetrapyrrole intermediates was detected. Neither did the loss-of-function mutants of the enzymes catalyzing the biosynthetic reactions from Proto to divinyl protochlorophyllide (Pchlide) show any changes in photosynthetic gene expression, as would have been predictable from the model.^{3,8} Nevertheless, results obtained upon incubation of algal and plant cells with porphyrins speak for the involvement of tetrapyrrole pathway on the chloroplast retrograde signaling.

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In *Chlamydomonas*, the treatments of cells with MgProto and MgProto methyl ester (MgProtoME) substituted for light in the induction of nuclear *HSP70s* and *HEMA* genes encoding chaperones and glutamyl-tRNA reductase of tetrapyrrole pathway, respectively.^{13,14} von Gromoff et al.¹⁵ identified a cis-acting sequence in the promoter region of *HSP70A* that is employed for induction by both MgProto and light in *Chlamydomonas*. Furthermore, a recent elegant paper by Kobayashi et al.¹⁶ provides substantial experimental evidences for regulation of nuclear DNA replication by a tetrapyrrole signal in plant cells. It was shown that in synchronized cultures of unicellular red alga and tobacco BY-2 cells, the organelle DNA replication preceded and was a prerequisite for nuclear DNA replication in the cell cycle. Additions of Proto and MgProto, however, substituted for the requirement of organelle DNA replication before the nuclear DNA replication. They also showed that organelle DNA replication, as well as porphyrins in the absence of organelle DNA replication, activated an A-type cyclin-dependent kinase that controls the nuclear DNA replication in the G₁-S phase transition of the cell cycle. These reports indicate that plastidial retrograde signals may not coordinate only nuclear genes for the photosynthetic machinery but also the cell cycle and stress responses in plant cells.

Distorted Chlorophyll Biosynthesis Causes Modifications in Nuclear HSP Gene Expression and Cell Division

Our studies on knockout lines of nuclear gene encoding chloroplast NADPH-thioredoxin reductase (NTRC) give support to the role of porphyrins as retrograde signals in Arabidopsis.¹⁷ Thioredoxin reductases are members of cellular thioredoxin systems that control the dithiol-disulphide exchange in proteins.¹⁸ Besides the severe defects in growth, the *ntrc* knockout lines had strongly reduced chlorophyll content in leaves.¹⁷ It is thus conceivable that NTRC regulates the tetrapyrrole pathway in chloroplasts, and that the knockout of this enzyme modifies the flux through the biosynthetic pathway from Proto to Pchlide (Fig. 1). Further, we propose that the imbalanced production of tetrapyrrole intermediates induces a plastidial signal that modifies nuclear gene expression in *ntrc* plants either directly or indirectly via production of ROS¹⁷ (Fig. 1B). This assumption is based on the regulatory mechanisms of the first enzymes in chlorophyll biosynthetic branch and on the structural and biochemical phenotypes of knockout *ntrc* plants. These aspects can be summarized by the following points. (i) Feeding of 5-aminolevulinic acid to

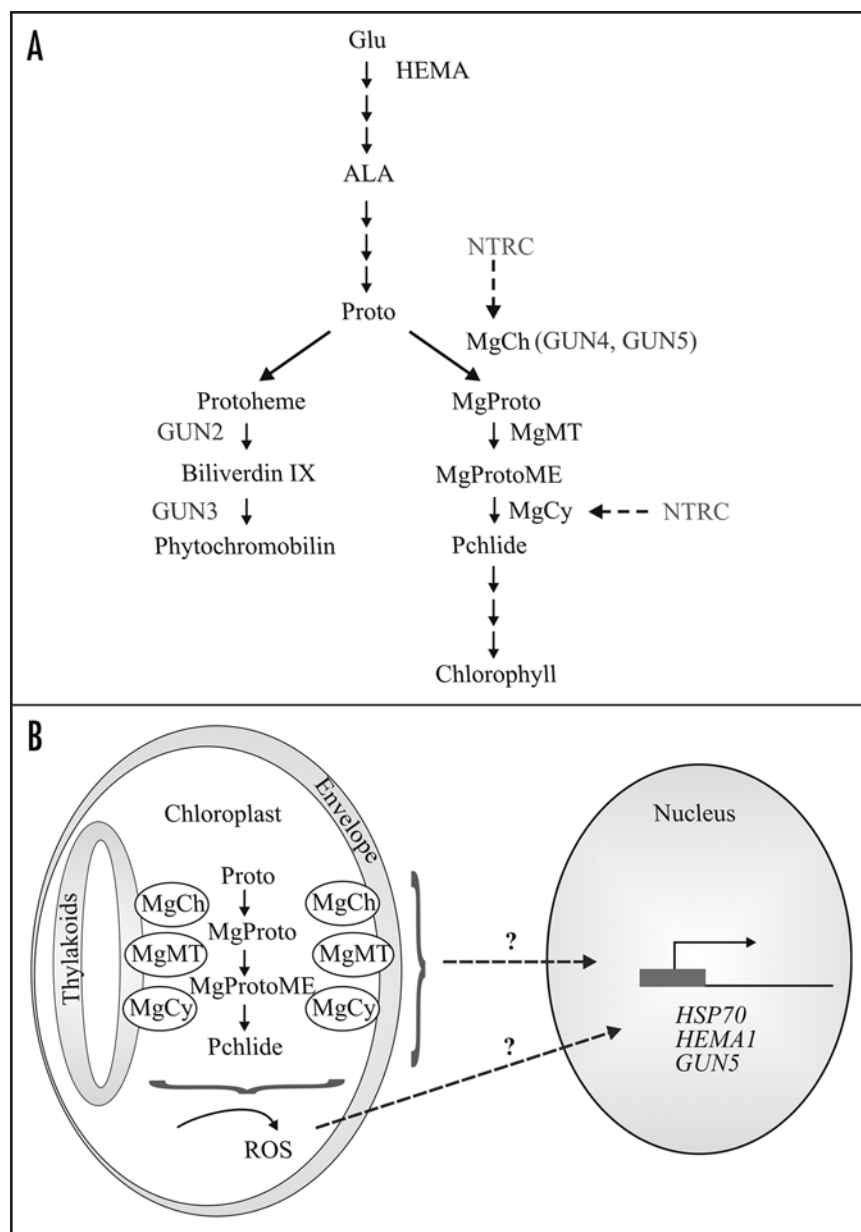


Figure 1. (A) Schematic pathway of tetrapyrrole biosynthesis in higher plants. The enzymes and *gun* mutants discussed in the text are marked in the pathway. Glu, glutamate; ALA, 5-aminolevulinic acid; Proto, protoporphyrin IX; MgProto, Mg-protoporphyrin; MgProtoME, Mg-protoporphyrin monomethyl ester; Pchlide, divinyl protochlorophyllide; HEMA1, glutamyl-tRNA reductase; MgCh, Mg-chelatase; MgMT, MgProto methyltransferase; Mg-Cy, Mg-protoporphyrin IX-monomethyl ester cyclase. (B) The tentative model for chloroplast retrograde signaling involving intermediates of chlorophyll biosynthesis. The porphyrin intermediates between protoporphyrin IX and protochlorophyllide may initiate the retrograde signal controlling the gene expression in nucleus. Alternatively, the intermediates may induce production of ROS that in turn activates the signaling cascade. For other details, see the text.

Arabidopsis in darkness resulted in increased accumulation of Proto, MgProto and MgProto methyl ester and reduced amount of Pchlide in *ntrc* compared to wild type,¹⁹ suggesting an imbalanced flux through Proto to Pchlide in *ntrc* leaf cells. (ii) This may result from reduced activity of Mg-chelatase in the absence of NTRC because this enzyme is regulated via thioredoxin-mediated disulphide/dithiol exchange.²⁰ Furthermore, in the presence of

chloroplast 2-Cys peroxiredoxin NTRC has been shown to stimulate the activity of Mg-protoporphyrin IX-monomethyl ester cyclase (MgCys), the enzyme catalyzing the reaction of chlorophyll biosynthesis downstream from Mg-chelatase.¹⁹ Interestingly, these enzymes together with MgProto methyltransferase are dually localized both in thylakoid membranes and in the inner chloroplast envelope membrane (reviewed in ref. 2), which provides a natural and rapid route for transporting signals from plastid to the cytoplasm. (iii) Four nuclear genes encoding enzymes in the tetrapyrrole pathway (including *GUN5* and *HEMA1*) and six genes encoding cytoplasmic heat shock proteins were found to be upregulated in *ntrc* leaves.¹⁷ *GUN5* transcripts, encoding the H subunit of Mg chelatase, showed the highest differential accumulation in *ntrc* line compared to wild type. *HEMA* gene and genes encoding HSP70 proteins were also induced in *Chlamydomonas* after treatment with MgProto and MgProtoMe in darkness.^{13,14} The *Arabidopsis* genome contains several *HSP70* genes encoding cytoplasmic HSP70 proteins, but it is intriguing to note that the upregulated genes in *ntrc* (*At3g12580* and *At3g09440*) encode cytoplasmic proteins that are the closest homologues to the single cytoplasmic *HSP70A* gene in *Chlamydomonas*.²¹ (iv) The pale green *ntrc* leaves have higher number of cells per area than wild type *Arabidopsis* and significantly reduced cell size with low number of chloroplasts,¹⁷ suggesting that the plastidial and nuclear divisions are uncoupled in *ntrc* plants. This might be explained by the substitution of plastidial DNA replication with tetrapyrrole signal in the control of nuclear division, like suggested recently by Kobayashi et al.¹⁶

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