A Mutant of the Cyanobacterium *Synechocystis* sp. PCC 6803 Lacking Geranylgeranyl Reductase as an Object for Studies of Photosynthesis and a Candidate for Production of Valuable Medical Compounds

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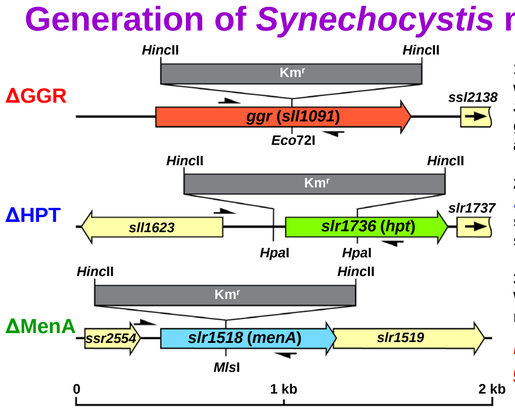
In the oxygenic phototrophs, *i.e.*, plants, algae, and cyanobacteria, geranylgeranyl reductase (GGR) catalyzes the stepwise reduction of geranylgeranyl to phytyl, the tetraprenoid moiety of chlorophyll, α-tocopherol (major constituent of vitamin E fraction), and phylloquinone (vitamin K_1). The *ggr* gene (ORF *sll1091*) encoding this enzyme was inactivated in the cyanobacterium *Synechocyctis* sp. PCC 6803. The resulting ΔGGR (formerly *ΔChlP* [1]) mutant exhibits pleiotropic phenotype that includes such notable properties as inability to grow photoautotrophically, sensitivity to increased illumination, and high abundance of phycobilisomes. Meanwhile, ΔGGR possesses active PSI and PSII providing efficient light-driven electron transport. For discrimination between impacts of affection of chlorophyll, tocopherol, and phylloquinone biosyntheses on the ΔGGR phenotype, two additional *Synechocystis* mutant strains lacking either homogentisate phytyl/prenyl transferase (HPT) specific for tocopherol biosynthesis (ΔHPT strain) or 1,4-Dihydroxy-2-napthoate phytyl/prenyl transferase (MenA) specific for phylloquinone biosynthesis (ΔMenA strain) were generated, and the ΔGGR mutant was compared with them in different experiments.

Generation of *Synechocystis* mutant strains



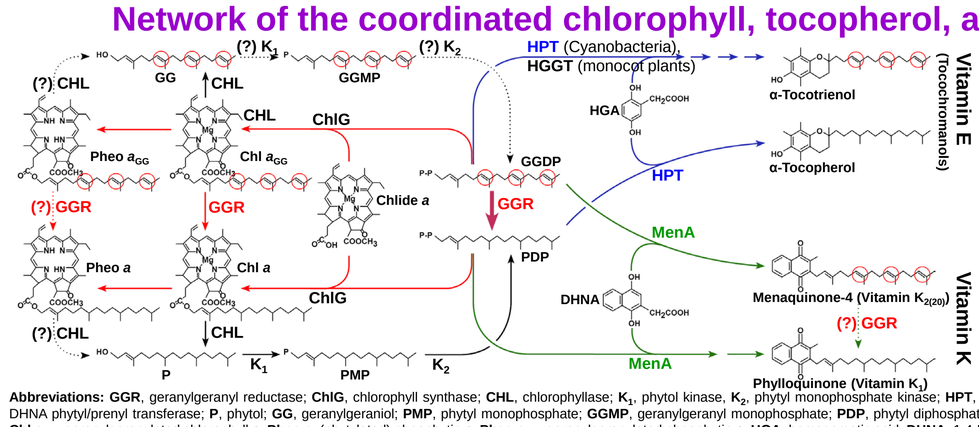
1) No paralogs of every gene were found in the *Synechocystis* sp. PCC 6803 genome by bioinformatics analysis.

2) The ΔGGR, ΔHPT, and ΔMenA mutants were segregated to the homozygous state.

3) Spontaneous restoration of WT phenotype in the ΔGGR mutant is impossible indicating

indispensable role of the ggr gene.

Network of the coordinated chlorophyll, tocopherol, and phylloquinone biosyntheses



Biosynthesis pathways affected in the constructed mutant strains of *Synechocystis* sp. PCC 6803	Chlorophyll *a* & Pheophytin *a*	Tocopherol	Phylloquinone
ΔGGR	Yes	Yes	Yes
ΔHPT	No	Yes	No
ΔMenA	No	No	Yes

Abbreviations: **GGR**, geranylgeranyl reductase; **ChlG**, chlorophyll synthase; **CHL**, chlorophyllase; **K_1**, phytol kinase, **K_2**, phytyl monophosphate kinase; **HPT**, homogentisate phytyl/prenyl transferase; **HGGT**, homogentisate geranylgeranyl transferase; **MenA**, DHNA phytyl/prenyl transferase; **P**, phytol; **GG**, geranylgeraniol; **PMP**, phytyl monophosphate; **GGMP**, geranylgeranyl monophosphate; **PDP**, phytyl diphosphate; **GGDP**, geranylgeranyl diphosphate; **Chlide *a***, chlorophyllide *a*; **Chl *a***, (phytylated) chlorophyll *a*; **Chl a_{GG}**, geranylgeranylated chlorophyll *a*; **Pheo *a***, (phytylated) pheophytin *a*; **Pheo a_{GG}**, geranylgeranylated pheophytin *a*; **HGA**, homogametic acid; **DHNA**, 1,4-Dihydroxy-2-naphthoic acid.

Physiological characteristics vs. WT

Parameter	ΔGGR	ΔHPT	ΔMena
Photoautotrophic growth (no glucose) at 40 µmol photon m^{-2} s^{-1} (moderate light)	NO	Like WT	Reduced
Photomixotrophic growth (with glucose) at 2-4 µmol photon m^{-2} s^{-1} (low light)	Like WT	Like WT	Reduced
Photomixotrophic growth (with glucose) at 40 µmol photon m^{-2} s^{-1} (moderate light)	Like WT	Like WT	Reduced
Photomixotrophic growth (with glucose) at 100 µmol photon m^{-2} s^{-1} (increased light)	NO	Slightly reduced	NO
Chlorophyll content (photomixotrophic growth in moderate light)	Reduced	Like WT	Reduced
Total carotenoid content (photomixotrophic growth in moderate light)	Reduced	Like WT	Reduced
Whole chain-mediated oxygen evolution rate (measured under saturating white light)	Enhanced	Like WT	Reduced

ΔGGR possesses active PSI and PSII of high electron transfer capacity but unable to grow photoautotrophically, however, and is high-light sensitive.

Chlorophyll and tocochromanol analyses by high-performance liquid chromatography (HPLC)

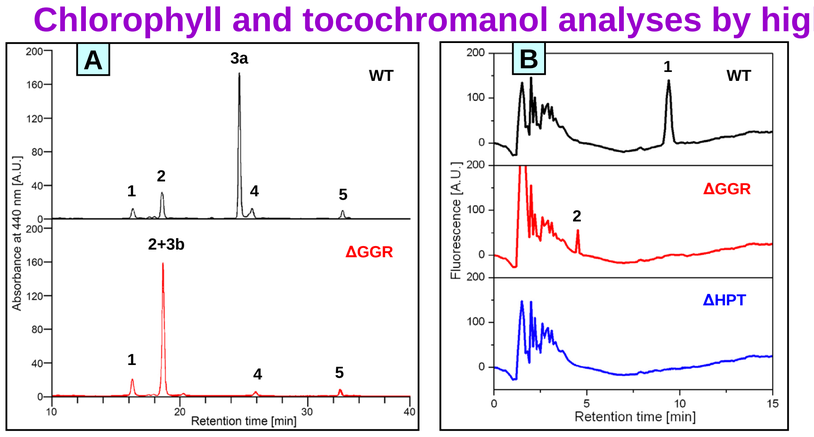


Fig. A. HPLC analysis of chlorophyll and carotenoid composition

1 – myxoxanthophyll,

2 – zeaxanthin,

3a – (phytylated) chlorophyll *a* (Chl *a*),

3b – geranylgeranylated chlorophyll a (Chl a_{GG}),

4 – echinenone,

5 – β-carotene

1) Normal (phytylated) chlorophyll *a* is completely replaced with its geranylgeranylated counterpart in the ΔGGR cells.

2) While total carotenoid level is decreased, the amount of myxoxanthophyll is increased in ΔGGR that might be indicative for oxidative stress in the mutant.

Fig. B. HPLC analysis of tocochromanols (Vitamin E)

1 – α-tocopherol

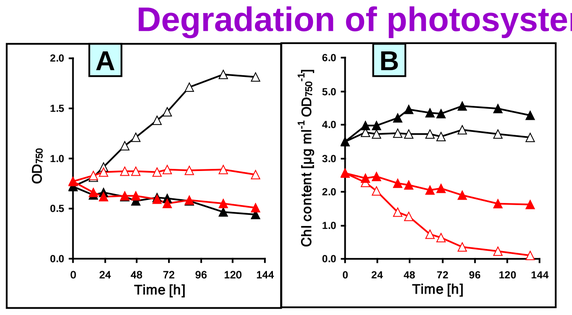
2 – α-tocotrienol

1) α-tocopherol is completely replaced with α-tocotrienol in the ΔGGR cells.

2) No tocochromanols (Vitamin E) are observed in the ΔHPT mutant.

Biochemical analyses confirmed absence of paralogs of the ggr and hpt genes in the Synechocystis sp. PCC 6803 genome and the complete inactivation of this genes in the respective mutant strains.

Degradation of photosystems



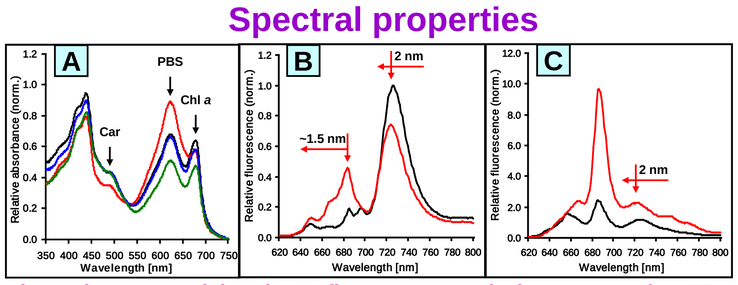
Cell growth (A) and chlorophyll contents (B) during incubation without glucose in moderate light and in darkness

WT, Light
WT, Dark
ΔGGR, Light
ΔGGR, Dark

The strains were grown photomixotrophically in moderate light up to a mid-log phase and transferred into a fresh glucose-free medium for incubation at the same light and in a full darkness.

Chl a_{GG} destabilizes photosystems (PSs), and their steady-state level can be retained only in case of extra energy source, e.g., glucose. Light enhances PSs degradation rate. Thus, GGR is crucial for photoautotrophic survival.

Spectral properties

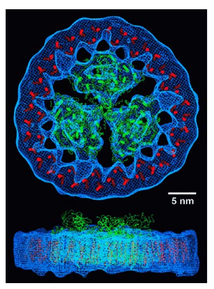


Absorption spectra (A) and 77 K fluorescence emission spectra of whole cells recorded upon Chl excitation at 435 nm (B) and upon excitation of phycobilisomes (PBS) without glycerol at 590 nm (C)

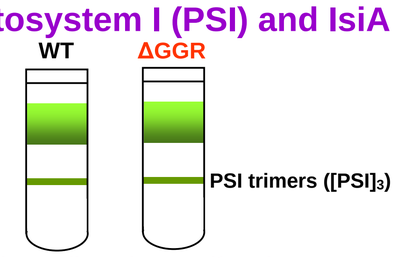
WT
ΔGGR
ΔHPT
ΔMenA

ΔGGR has lower levels of Chl and carotenoids (Car), decreased content and perturbed structure of the PSI. The PBS are abundant and transfer energy to the PSs; however, possibly a portion of PBS in ΔGGR is energetically not tightly coupled to the PSs giving high fluorescence yield from the terminal emitter. Thus, *Chl a_{GG} perturbs photosynthetic apparatus.*

Determination of photosystem I (PSI) and IsiA



IsiA Chl-protein complex around PSI trimer (adapted from Ref. [2])



Sucrose density gradient ultracentrifugation

Chl a_{GG} enables assembly of PSI into trimers. Meanwhile, no [PSI]$_3$-IsiA complex is formed in the ΔGGR mutant under moderate light, which is stressing for the mutant, anyway. That implies other mechanisms that could partially compensate the deleterious impact of light on the photosynthetic apparatus in ΔGGR.

Impact of high irradiance

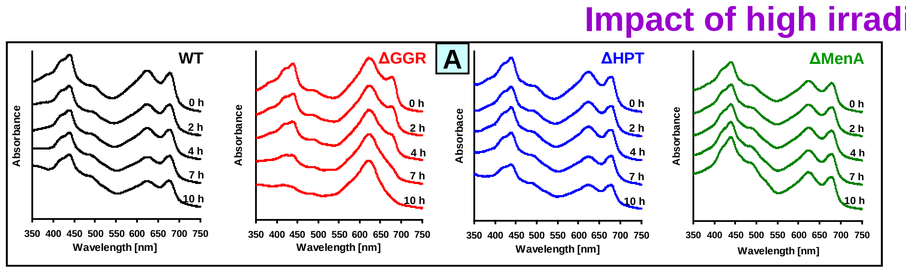


Fig. A. *In vivo* absorption spectra. The spectra were recorded at different time points (0, 2, 4, 7, and 10 hours) of incubation at 500 µmol photons m^{-2} s^{-1} (high light) in the presence of glucose. Only slight pigment decrease was observed in the WT and ΔHPT strains. Despite of sensitivity to increased illumination, pigments are stable in the ΔMenA strain in accordance with the data reported [3]. In contrast, rapid chlorophyll decay indicative for degradation of PSs is observed in the ΔGGR strain. From the other hand, the abundant phycobilins are not down-regulated in ΔGGR that implies inhibition of protease activity responsible for phycobiliprotein degradation.

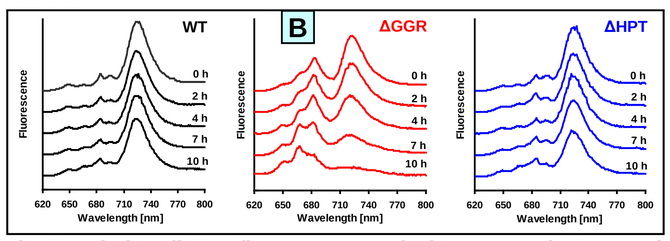
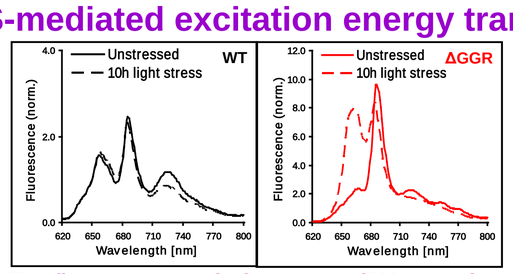


Fig. B. Whole cell 77 K fluorescence emission spectra (435-nm ex.)
The spectra correspond to the samples, for which absorption spectra were obtained (Fig. A). Fluorescence was recorded upon ecxiation at 435 nm in the presence of glycerol. WT and ΔHPT demonstrated identical fluorescence pattern. Unlike that, rapid decrease of the PSI emission peak corroborating absorption spectroscopy data and the perturbing fluorescence in the PSII-PBS related region are observed in ΔGGR.

1) Rapid degradation of PSs under high-light radiation is one of the major reasons of high-light sensitivity in the ΔGGR mutant strain.
2) Neither lack of tocopherol nor lack of phylloquinone but Chl a_{GG} is the major factor responsible for high-light sensitivity of PSs in ΔGGR.

PBS-mediated excitation energy transfer



77 K fluorescence emission spectra (590-nm ex.)

The samples were measured without glycerol. Unstressed cells (solid lines) and high-light stressed cells (broken line) were analyzed. The spectra were recorded on the samples taken from the light-stress experiment (0h and 10h time points) presented in the panel 'Impact of high irradiance'.

In the intact ΔGGR cells, excitation energy transfer from PBS to PSs, in particular to PSI, occurs. In the high-light treated cells, PSs degradation is accompanied by accumulation of PBS energetically decoupled from thylakoid membrane. Such PBS loose their native internal structure.

Conclusions: **Among oxygenic phototrophs, the ΔGGR mutant is the first example of the full inactivation of the GGR function [1, 4, 5]. Comparison with the tocopherol and phylloquinone deficient *Synechocystis* strains revealed that inability of ΔGGR to retain PS steady-state level under photoautotrophic and high-light conditions specifically relates to Chl a_{GG}. Besides, specific perturbations of the photosynthetic apparatus in ΔGGR were shown. From the other hand, the data demonstrated that Chl a_{GG} does not prevent assembly of functionally active PSI and PSII. Additionally, in contrast to the phylloquinone deficient mutant (present work and published by Johnson *et al*., 2000 [3]), high rate of electron transfer through the whole chain implies replacement of phylloquinone with menaquinoe-4 in the mutant's PSI A_1 site in analogy with the reported rice mutants with the affected geranylgeranyl reductase [6]. Collectively, the data indicate that phytyl structure of Chl *a* tail and, thus, GGR are extremely important for PS stability and acclimation to the photosynthetically necessary light intensities in the oxygenic phototrophs.**

Prospects: **The obtained results are of interest for both pure photosynthesis research and practical applications. In particular, the pure research assume fine investigations of Chl-protein, Chl-Chl and Chl-Car interactions mediating assembly of photosynthetic pigment-protein complexes, excitation energy transfer and electron transfer processes. In this respect, construction of the double or triple mutants additionally lacking PSI, PSII, PBS (G1, G2, or both forms), or some other photosynthetically important systems is a promising approach. In the meaning of practical applications, the ΔGGR mutant can be considered as a candidate for production of compounds that have or might have certain medical values. They include (*i*) menaquinoe-4 (MK-4 form of vitamin K_2) controlling bone development, blood clotting, and decalcification of blood vessels in human; (*ii*) Chl a_{GG}, which might possesses certain advantages vs. the phytylated counterparts used in the photodynamic cancer therapy; and (*iii*) α-tocotrienol along with CPC as specific antioxidants. It should be noted that the respective plant mutants cannot be useful in this respec due to inability to survive beyond the seedling stage, whereas the cyanobacterial ΔGGR mutant can be successfully propagated photomixotrophically in bioreactors for providing ample biomass.**

References:

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